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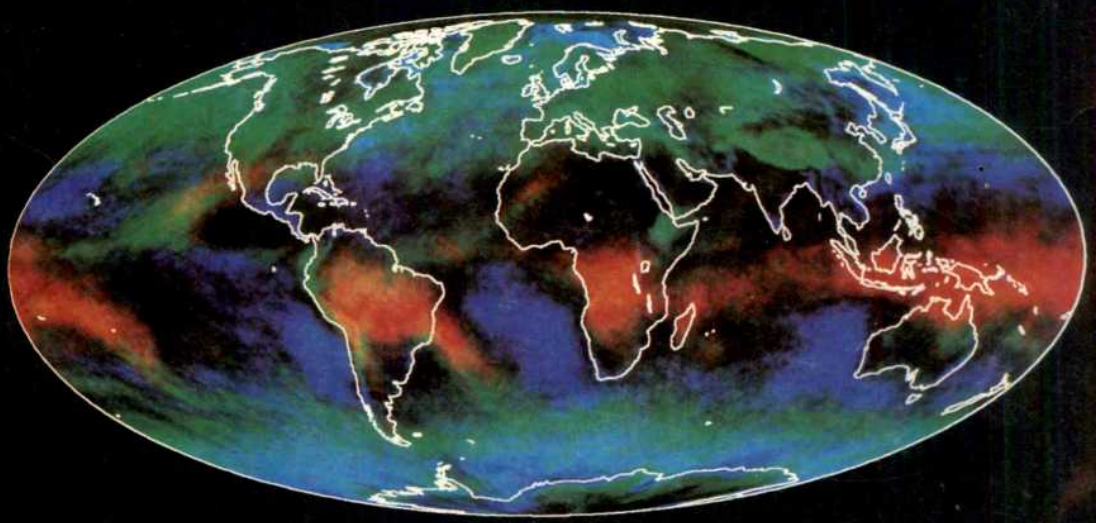

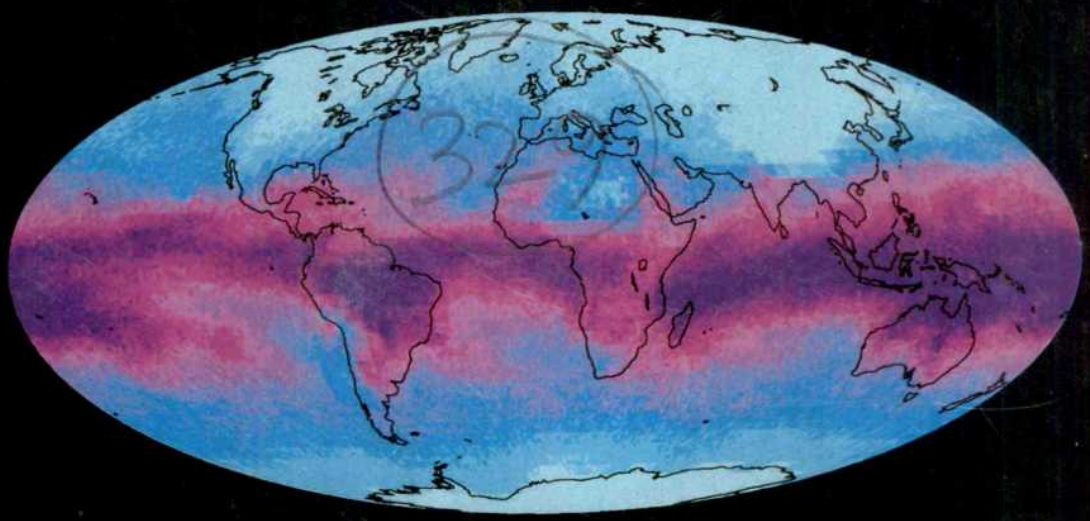

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**Resolving circumstellar disks**

**Beta-sheet DNA motif**

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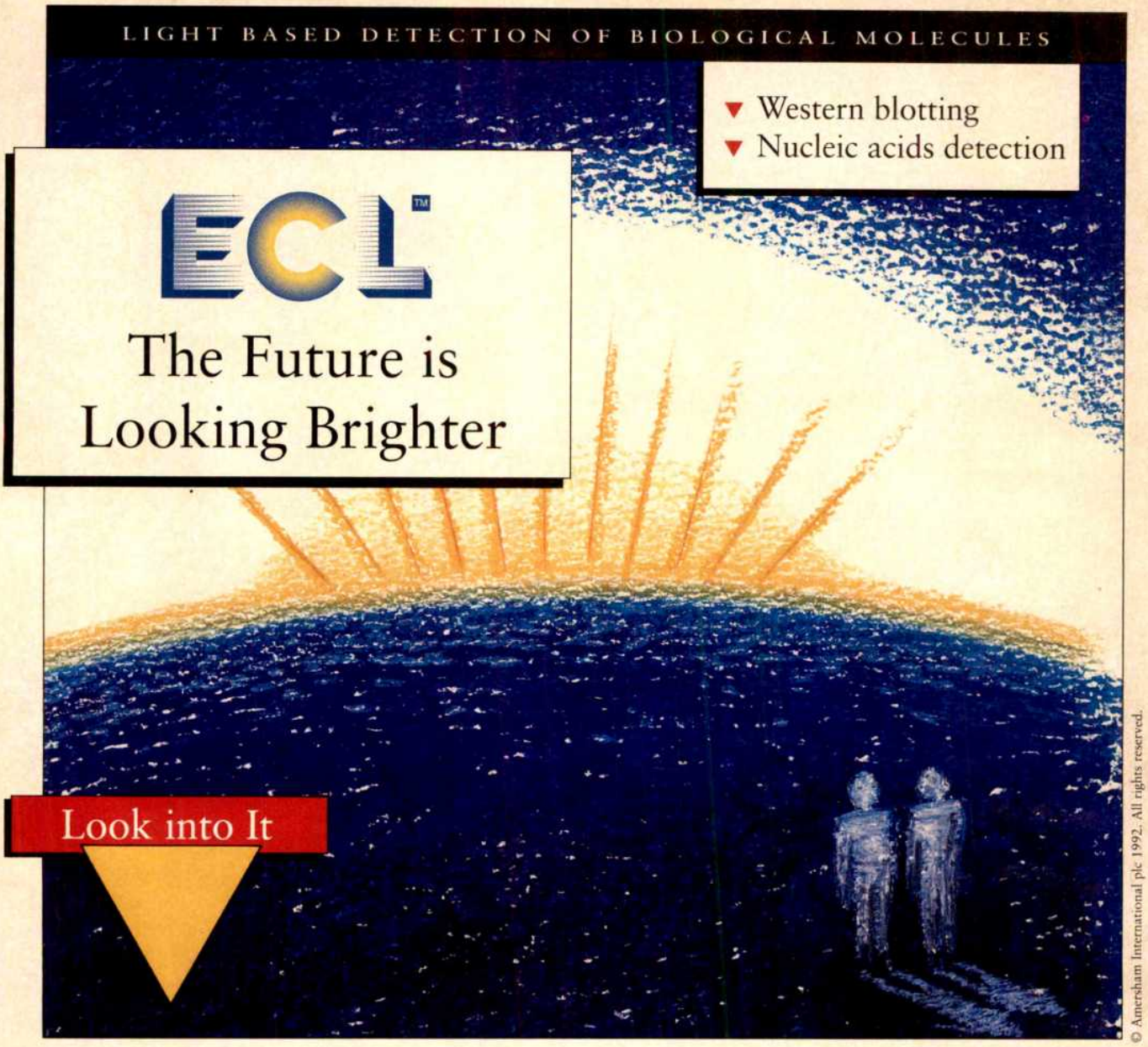




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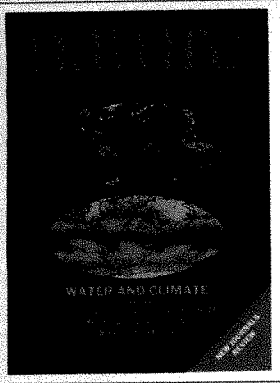



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**1 October 1992**
**Vol. 359 Issue no. 6394**


◀ The upper image shows mean cloud cover and altitude (highest clouds in red, lowest in blue); the lower shows mean precipitable water vapour, both for January 1979. The correlation between high clouds and intense evaporation (dark purple) in the tropics provides one of many links between the hydrological cycle and climate discussed in this week's Review Article by Chahine, page 373.

## THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

### Chromosome 21
The first complete contiguous map for the long arm of human chromosome 21 is presented on page 380. This set of overlapping clones, derived using the YAC (yeast artificial chromosome) method which is showing promise in the Human Genome Project, covers the chromosome associated with a number of genetic diseases, including Down's syndrome, some forms of Alzheimer's disease and progressive myoclonus epilepsy. News and Views page 367.

### Cloud cover
High-resolution polarimetry of two pre-main-sequence stars in the same star-forming cloud shows them both to be surrounded by substantial disks of dust and gas, comparable in size to our Solar System. This finding suggests that such potentially planet-forming clouds could be a common feature of stellar evolution. Pages 399 and 360.

### Beta recognition
A two-stranded β-sheet would be an ideal protein motif for specific interaction with DNA, yet the main classes of DNA binding proteins seem to use α-helices to contact their target sequences. Somers and Phillips (page 387) present the first detailed structure of a protein/DNA complex where the specific interaction is mediated by a β-sheet motif, and He *et al.* (page 431) use site-directed mutagenesis to examine the repressor–operator interaction.

### White alert
Is the cultivar 'blanche fleur' of *Vicia sativa* being exported from Australia for human consumption in poorer countries? Reports that the legume contains potentially dangerous levels of neurotoxins means that its use as a food should be banned until reliable data have been collected and, if appropriate, the plant modified either genetically or by post-harvest processing. Commentary, page 357.

### Positive trend
Six years of monitoring of the atmospheric concentrations of the halons H-1301 and H-1211, which have been used for 30 years in fire extinguishers, reveals a decrease in the growth rates of these two ozone-destructive halons which is consistent with industry emission estimates. Manufacture of H-1301 and H-1211 is due to be discontinued by the year 2000, but signs are that with continued voluntary reduction in use during the next few years, the atmospheric levels of these pollutants should stabilize, or even decrease. Page 403.

### Father figures
Mitochondrial DNA is often assumed to be inherited matrilineally, despite some evidence for slight paternal leakage in several species. On page 412, though, Zouros *et al.* demonstrate significant paternal leakage of mtDNA in two species of the mussel *Mytilus*, showing that the phenomenon transcends curiosity status and must be addressed seriously in phylogenetic studies using mtDNA.

### Better than diamond
Synthetic diamond films may find application in microelectronic circuits, spreading the heat produced by tightly packed electronic devices to prevent thermal damage. On page 401, Graebner *et al.* measure the thermal conductivity near the top growth surface of a synthetic diamond film and find that, surprisingly, it is at least as high as that in gem-quality diamond single crystals.

### Leaving the nest
Sex-biased juvenile dispersal may function to separate opposite-sex relatives to avoid incestuous mating, or same-sex relatives to avoid reproductive competition. On page 409, Wolff reports that the presence of opposite-sex parents suppresses reproduction and stimulates dispersal of juvenile white-footed mice to avoid inbreeding.

## NATURE SAYS



## NATURE REPORTS



## CORRESPONDENCE



## COMMENTARY



## NEWS AND VIEWS



## SCIENTIFIC CORRESPONDENCE



## NEW JOURNALS REVIEW



This issue contains *Nature*'s annual review of new journals. Reviews begin on page **436**, with an index on page **437** and a list of other journals received on page **464**

## REVIEW ARTICLE




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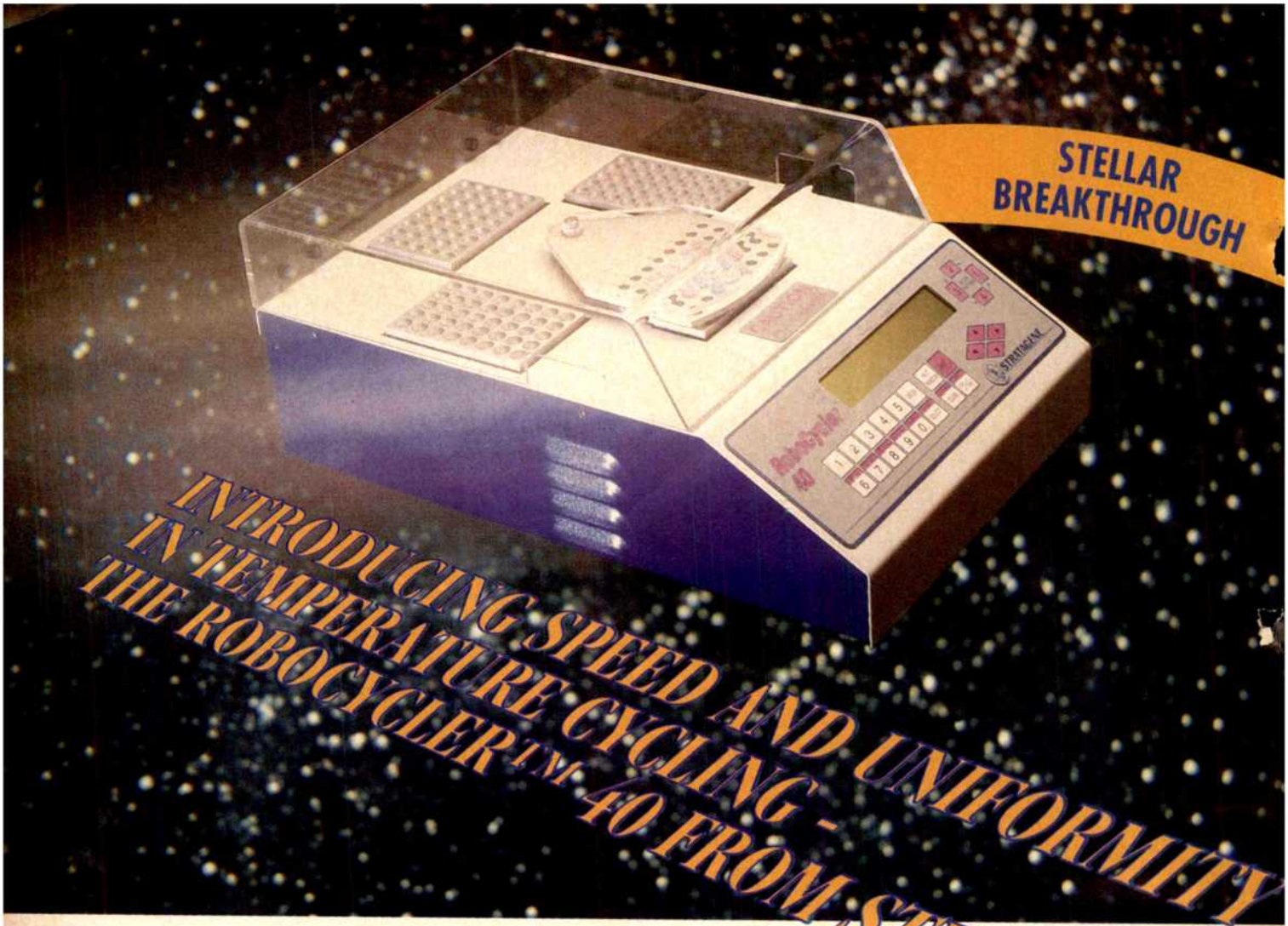


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## NATURE GENETICS



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# Europe's recalcitrant offshore island

**The chauvinistic response of many Britons to the events of the past two weeks would be better directed towards an understanding of the weakness of British industry — the real reason why £1 is no longer worth DM2.95.**

BRITAIN, the largest of the European offshore islands, will not easily recover from the shocks of the past few weeks. The government seems indeed to be following the tactical course of doing without a European policy of its own until Denmark's objections to the Maastricht treaty have been resolved (see *Nature* **359**, 257; 1992). It hopes that a start can be made on that at the special meeting of the European Council it has arranged on 7 October. Its difficulty is that the expulsion of the British currency from the European Exchange Rate Mechanism (ERM) and its subsequent devaluation have brought to the surface the doubts of all those who have never accepted that Britain is a part of Europe. The political pollsters guess that between a quarter and a third of the members of the House of Commons would vote against the Maastricht Treaty in its present form if the government gave them a chance. The longer the uncertainty persists, the greater will be the opposition. But what has changed in the past few months except that the foreign exchanges have declared that £1 is not worth DM2.95, but rather less?

## Surprise

Can anybody be surprised? Whatever the proper value of sterling when Britain joined the ERM late, two years ago, Britain's strength as a productive enterprise has since dramatically declined. There have been floods of bankruptcies among companies large and small. The disappearance of enterprises such as Robert Maxwell's publishing empire and the enforced devaluation by banks and other financial institutions of their balance sheets are not irrelevant to the value of sterling on the foreign exchanges. Nor is Britain's balance of payments, which may well exceed £10 billion in the year that ends next March; neither is the government's plan to borrow in excess of £25 billion during the same period just to pay its bills. There are, of course, many in Britain who hold that these misfortunes would not have come about if it had not been for Britain's membership of ERM, and for the recession either brought about or intensified by the attempt to maintain sterling at DM2.95. But that is not the case.

British politicians who believe otherwise need only pay attention to last week's further loss of skilled jobs in British manufacturing industry. After years of steady decline, the number of people now working in British manufacturing industry is roughly 5 million, but no fewer than 0.1 per cent of them were put out of work last week alone. Moreover, the sources of these job losses are significant. They included 600 people at Rolls-Royce Motors (not to be confused with the jet-engine builder of the same name), which makes hand-built cars of antiquated design for the international luxury market, and 2,500 people working for Britain's only surviving manufacturer of civil aircraft, itself a part of the recently privatized company called British Aerospace. (That enterprise seems certain to be closed if negotiations to form a Taiwanese partnership fall through.) Other motor manufacturers also continued to shed staff last week.

Often during the past decade it has been argued that such trends are irrelevant to Britain's economic future. Some have held that the decline of manufacturing would be more than counterbalanced by the growth of the business of providing financial services, from stockbroking to insurance. Sadly, that dream has been tarnished by a series of muddles (and worse) in the financial industry. There is nothing in the recent balance of overseas payments to suggest that the dream will come true in the near future. But then, other optimists have argued, high technology will help to save Britain's skin. Sadly again, that dream is also a long way from realization. It is true, of course, that several small companies founded on the exploitation of innovation have managed to avoid the wave of recent bankruptcies (although many more have been forced to the wall). But their rate of growth is nearly great enough to make up for the decline of more conventional manufacturing. And with the exception of the successful British pharmaceutical industry, larger companies have been conspicuously unwilling to risk doing things for the first time.

## Jeopardy

The implication is plain: Britain's future as a modern industrial economy is in jeopardy. That, no doubt, was one of the considerations the currency traders had in mind when they were selling sterling short two weeks ago. The then impending referendum in France on the Maastricht Treaty was merely an occasion when speculation might succeed. The injured pride with which many of the British have responded is understandable, but purposeless. Indeed, the logical response would have been to recognize that British industry's best chance of survival lies in still closer integration with the European market for goods and services. There is no escape from that. It is puzzling that the British government, knowing this, should have kept its silence on this issue since the debacle on the foreign exchanges.

In its own and its taxpayers' interests, the British government should now exert itself outside the fields of its present

preoccupations, largely with monetary and fiscal matters (and with endless recalculations of the likely strength of the parliamentary opposition to the Maastricht Treaty). To be sure, it will help the economy in general if interest rates are reduced, even if there will have to be higher taxes early in 1993 to pay for them. But the government should be even more concerned with removing present British impediments to modernity.

This is not to suggest that the government should again take a hand in the ownership and management of industry, but that it should exert its potentially powerful influence on the way in which British industry is managed, or mismanaged. After a decade in which British academic researchers were upbraided for their supposed indifference to economic imperatives, it is high time that British industry's managers were required to shoulder responsibility for the neglect of innovation (there are exceptions) and their preoccupation with the next half-year's bottom line rather than the long-term welfare of their enterprises (and of those who work in them). The government itself has direct responsibility for education and training in modern skills, in management as well as technology, but has neglected them. Will it now do better?

The government could also exert a beneficial influence on the temper of the British reaction to the events of the past few weeks, which has been tinged to an unhealthy degree with chauvinism, even xenophobia. While there are good reasons for asking that the Maastricht Treaty should not come into force without important (and negotiable) changes in the way in which the European Communities' democratic, administrative and legal institutions function, those in Britain now yearning for a return to the simplicities of island independence should more often be reminded of the heavy price in collective impoverishment that would have to be paid for that claustrophobic condition. The British prime minister, whose policy was to put Britain "at the heart of Europe", and who happens to be the current president of Europe, might say these things more often than he does. Whether they would lose votes is arguable, but does not public responsibility require no less? □

# Gene patents

**The decision of the US Patent Office not to protect gene fragments is welcome, but questions remain.**

Now for the good news! The rejection last week by the US Patent Office of the application by the US National Institutes of Health (NIH) for patents covering more than 2,000 human genes identified by cDNA cloning is thoroughly welcome (see *Nature* **359**, 263; 1992). For the past year, the possibility that researchers might obtain a licence for the exclusive exploitation of a gene which has not even been fully characterized, but merely made identifiable by means of a length of cDNA typically a few hundred bases long, has been the source of consternation in the human genome community. (The European Science Foundation is the research organization most recently to speak out against the prospect.) For the time being at least, the threat has faded.

Why does (or did) it matter? The simple explanation is that the description of the nucleotide sequence of the human genome must necessarily be an international venture. So much is plain from the mere sight of the list of authors at the head of almost any research article in the field (see, for example, *Nature* **359**, 380; 1 October 1992). Moreover, it is exceedingly difficult to understand how the venture could succeed without the free exchange and publication of information. NIH's original explanation of its application for patent protection for the 2,000 gene fragments was that free access to its information could be assured only in such a way. US patent law would indeed provide for that. But patent law elsewhere is different, and does not provide for a period of twelve months after publication during which patent applications are still possible. The unwelcome result of the NIH application was to persuade others to apply for patents first and to make data available only afterwards. That inhibition may now have been removed.

That, at least, is the reasonable expectation. Although NIH plans to appeal against the ruling by the Patent Office, and is talking as if it is confident of the outcome, there seems little room for further argument over the chief grounds on which the patent application was turned down. The best hope now is that the Patent Office will appreciate the need for a speedy resolution of the appeal, the mere existence of which may persuade others working in this field not to make their data publicly available. Indeed, a swift determination could be invaluable. When first announced, the mere possibility that it might be possible to patent even fragments of human genes seemed an affront to commonsense. The whole business has served to heighten public anxiety about the supposedly innate wickedness of geneticists. If the outcome of the appeal were to clarify what the Patent Office considers valid under the existing legislation, NIH's adventure may yet prove to have been worthwhile.

The question remains of where to draw the line on gene patents. The US Patent Office appears to have decided against NIH on the grounds that its purported inventions were "obvious". The question of the "utility" of the gene sequences is given only scant attention, but would no doubt have provided a further hurdle at which the application would have fallen. For what use, in itself, is the nucleotide structure of a gene when nothing is known of its function, in normalcy and disease? One might as well expect to be able to patent the knowledge of the umpteenth digit in the decimal representation of the quantity $\pi$. On the other hand, if a person has identified a gene and shown how it may be used for some practical purpose, the spirit of patent law suggests that he or she should be entitled to protection. That would, for example, apply when a method had been developed for using the knowledge of the gene to treat an inherited disease (as familial hypercholesterolaemia is now routinely dealt with). Those in the US Congress planning to amend the law so as to clarify the position should follow that rubric and not attempt to make patentable the next clutch of sequences of unknown function. □

# Courts reject DNA fingerprinting, citing controversy after NAS report

**Washington.** Four months after the National Academy of Sciences (NAS) released a report intended to end the controversy over DNA forensic fingerprinting, the situation is as muddled as ever. At least three state courts have ruled that DNA evidence is inadmissible despite the report's conclusion that it should be accepted. The situation has deteriorated to the point where one DNA fingerprinting company — the Maryland-based Cellmark Diagnostics Inc. — would like the NAS panel to clarify its conclusions.

The courts have rejected DNA evidence for reasons not foreseen when the report was released in April (see *Nature* **356**, 552; 1992). One of its recommendations was for better laboratory quality-control standards and certification procedures, and some DNA fingerprinting critics have argued (persuasively enough to appear on the front page of the *New York Times* in an article that was essentially retracted the following day) that DNA evidence should be inadmissible until such procedures are established.

But the issue that most bothered the courts relates to statistics, not standards. The California Court of Appeals, the Massachusetts Supreme Judicial Court and the US District Court of Guam have all ruled (citing the NAS report and the accompanying controversy) that the scientific uncertainty over the role of population substructure in calculating the chance of DNA matches is too great to pass the so-called *Frye* test, a measure of scientific acceptance needed for legal acceptability set out in a 1923 decision by the US Supreme Court. As a result, DNA evidence using all but the most conservative statistics is now inadmissible in Massachusetts, some districts of California and Guam.

Although the NAS panel thinks that the use of DNA evidence is appropriate, that message has struggled to get out. A day before the report was to be released, an article in the *New York Times* characterized its conclusions as striking a blow against DNA evidence. The chair of the panel, Victor McKusick of Johns Hopkins University, denied that interpretation at a hastily scheduled press conference a few hours after the article appeared. Since then, several other articles — most notably a June review by Richard Lewontin of Harvard University in the *New York Review of Books* — have argued that the NAS panel did not understand the implications of its own report.

The rulings in Massachusetts and California support that argument. The NAS report called for an expanded database drawn from population subgroups and recommended, until that exists, the use of a modified 'ceiling' principle in which geneticists would make very conservative judgements on the chance of a certain fingerprint being matched at random. Although the NAS committee did not say that evidence derived from different statistical methods should meanwhile be inadmissible, that is what some courts have concluded.

On 7 August, in *People v. Barney*, the California court blamed prosecutors for introducing evidence using a 'product rule' calculation that gives an extremely low chance (1 in 200 million) of a random match.


Noting that this technique "has not received general scientific acceptance", the court concluded that "no amount of after-the-fact fine tuning can cure the error, [which] infects the underlying evidence...." On 20 July, in *Commonwealth* v. *Lanigan*, the Massachusetts court ruled that "the national call for considered, conservative approaches to DNA testing, such as the use of ceiling frequencies, and the absence of such an approach in the present case underscore the wisdom" of rejecting DNA evidence in an earlier trial.

It is true that several states have accepted DNA evidence since the NAS report, and that several others, including Ohio, have passed legislation explicitly making DNA evidence admissible in paternity cases. But that lack of uniformity existed before the NAS report, and it is disappointing that the report has not resolved it.

For whatever reason, DNA evidence continues to be held to a higher standard than other scientific subjects. That standard, under *Frye*, requires only "general acceptance" in the scientific community. Yet DNA evidence is often being rejected on the basis of controversies involving a few scientists, including Lewontin, who proclaim themselves to be extremists. (They defend themselves on the grounds that lives are at stake.) Despite the NAS report's endorsement of the fundamental soundness of DNA evidence, its conclusions have apparently been obscured by this debate on the scientific fringe.

"It's clear that the message hasn't gotten through," says Mark Stolorow, manager of forensic services at Cellmark. But courts that have rejected DNA evidence have either misinterpreted parts of the report or focused on side issues, he says; they have not challenged its conclusions.

Much of the problem stems from prosecutors who continue to use methods such as the product rule to come up with extraordinarily low chances of random matches. "There is absolutely no need to come in with statements like 'one in a billion'", says Philip Reilly, a member of the NAS panel. "One in 10,000 is just as good."

Prosecutors seem to be catching on, however. Stolorow says that Cellmark is receiving an increasing number of requests to calculate DNA match statistics based on the ceiling technique as well as the usual product method. And the Federal Bureau of Investigation has started providing a ceiling calculation to prosecutors who request it, according to John Sylvester, chief of the agency's DNA legal assistance unit.

But more conservative numbers also pose a problem, Stolorow says: many scientists do not believe they provide an accurate measure. Testifying under oath, such a scientist might be forced to give his or her professional opinion on the statistics question. If, in the process of advocating more extreme statistical techniques, an expert witness casts doubt on the NAS report's more conservative conclusions, the DNA evidence could be rejected on the grounds that there is insufficient "scientific agreement" on the issue.

Most people agree that the current confusion is temporary and that the introduction of DNA evidence will someday become routine in the courtroom. But many of the scientists who follow the issue are disappointed that the NAS study did not resolve it. Cellmark wants the panel members to draft a letter discussing — and presumably rejecting — some of the recent court decisions. But the NAS committee has formally disbanded, making that unlikely.

**Christopher Anderson**

# Report proposes US forum to link research to needs

**Washington.** Long-range planning is all the rage among US science policy-makers, and the prize for the most ambitious proposal goes to the Carnegie Commission on Science, Technology and Government, which has this week proposed a national forum to seek a consensus on the country's goals for science and technology. The forum would be based at the US National Academy of Sciences (NAS) and keep its eye on the 'big picture' — what the government should expect from its investment in science.

The idea is outlined in the commission's latest report*, released on Wednesday (30 September). It is in tune with the wishes of US Representative George Brown (Democrat, California) and his House science committee, which sponsored a press conference to announce the report, and comes as the directors of the National Institutes of Health and the National Science Foundation are each preparing reports on their agency's future (see *Nature* **359**, 261 & **358**, 355; 1992).

The field may already seem crowded with such bodies. To mention just a few, there is the Office of Science and Technology Policy and the President's Council of Advisors on Science and Technology, working on behalf of the executive branch; the Office of Technology Assessment (OTA), serving Congress and each of its myriad committees that oversee slices of the research pie; and the NAS itself, offering impartial advice to whichever government body requests it. But this group would be different, insists the chairman of the Carnegie panel, H. Guyford Stever. "The objective is to bring together professionals in science and engineering with people outside the field that nevertheless have a deep interest in how science affects society", he says. "I'm not sure that such a thing is occurring anywhere else."

Carnegie officials have been airing the idea for more than a year and are pleased to hear some of their ideas in the current debate about the best way to shift the rationale for supporting science from fighting the Cold War to serving the civilian economy and the needs of society. The academy is believed to be drawing up plans for such a national forum, which Stever estimates would cost $1 million a year to do properly, and a strong leader is considered vital to its success. Fortunately, there is no shortage of eligible candidates, among them Frank Press, who retires next July as NAS president, and John Gibbons, a member of the Carnegie task force that produced the report, who is thought to favour early retirement from his position as director of OTA. **Jeffrey Mervis**

* *Enabling the Future: Linking Science and Technology to Societal Goals* (Carnegie Commission on Science, Technology and Government, 10 Waverly Place, New York, NY 10003; telephone (212) 998-2150.)

# Agencies told to transfer technology — or else

**Washington.** A group of industry leaders last week called for a package of new incentives and pressures to force the US national laboratories to do a better job of transferring their technology to industry. In a new report*, the privately financed Council on Competitiveness calls on the government to earmark at least 10 per cent — about $1 billion — of the laboratory budgets of the Department of Energy (DOE) and National Aeronautics and Space Administration to technology transfer programmes and to take away that money if certain milestones are not reached.

The report is only the latest proposal to focus the national laboratories on helping US industry instead of on winning the Cold War. But it is the first to suggest potential cuts and still be endorsed by the agencies themselves.

Unlike some previous reports (see *Nature* **356**, 372 & 353, 578; 1992) the new study does not call for a separate technology transfer centre or company. In particular, the panel rejected the idea of turning one of the DOE laboratories into a technology outlet for the others, on the grounds that having such a clearinghouse would further remove researchers from the marketplace.

In other recommendations, the panel said that laboratory directors should be allowed to negotiate with industry without going through DOE headquarters and direct in-house research towards specific needs. It also urged the government to set goals that, if not reached in 3–5 years, would result in the money being redirected to more profitable research ventures elsewhere.

However, the panel stopped short of recommending that the laboratories be forced to make up a portion of their budgets with matching funds from industry. That proposal, first aired by the White House Council on Competitiveness, was rejected by everyone from laboratory directors to the companies themselves.

**Christopher Anderson**

* *Industry as a Customer of the Federal Laboratories* (Council on Competitiveness; 1992)

# Don't ask for too much, industry tells Congress

**Washington.** US high-technology companies do not want the federal government to demand useful products from the research that it funds, seven corporate executives told the US Congress last week. Instead, the government should continue on its present course of training a scientifically literate population and increasing the sum of knowledge in the world.

The occasion was a hearing before the science subcommittee of the House Committee on Science, Space and Technology, whose chairman, US Representative George Brown (Democrat, California), has talked recently about ending "the free ride for science" in the federal budget (see *Nature* **359**, 175; 1992). If scientists cannot help to strengthen the US economy, according to one popular refrain, then perhaps they do not deserve to be funded. But what the subcommittee heard from the likes of Arden Bement of TRW Inc., Robert Frosch of General Motors and Theodore Cooper of the Upjohn Company was that the government, in the words of Edward Penhoet of Chiron Corporation, "should do what industry cannot do — education and basic research".

That view is, to be sure, self-serving. Industry enjoys being free to pick and choose from among the best that government-funded scientists can offer, turning basic research into products as the opportunity arises, and would rather not be burdened with a meddlesome Congress or federal agency constantly telling it how to invest its resources. But leaving to industry the task of generating wealth also has its benefits: mistakes are the responsibility of the company, not the government, and success is supposed to come to those who know what the public wants and can deliver it.

What the corporate executives want most from the government is the proverbial 'level playing field' with their foreign competitors and an end to troublesome regulations that, as Cooper described them, have the Nuclear Regulatory Commission telling companies to lock the doors of laboratories using radioisotopes at the same time as the Occupational Safety and Health Administration prohibits locks on the doors. They also want to have a voice in shaping forthcoming government decisions affecting research, with Bement complaining about a lack of industrial input in half-a-dozen recent initiatives in such areas as high-performance computing, advanced manufacturing and biotechnology. What they fear is a government determined to create a central plan for research that goes into great detail about the expected return in any number of critical fields. **Jeffrey Mervis**

# Merck promises large grant to university if Canada extends patent protection

**Quebec.** A subsidiary of the world's largest drug company has pledged C$15 million (US$12 million) for Canada's first molecular medicine and therapeutics centre if the parliament approves legislation to extend patent protection for new drugs. The legislation, expected to be passed by the end of the year, is the government's reward to the pharmaceutical industry for more than doubling its research and development spending since the last extension in patent law was granted in 1987 (see *Nature* **355**, 666; 1992); the grant from Merck Frosst Canada Inc. is the biggest single investment by industry in university-based biomedical research in Canada.

Canadian pharmaceutical companies have announced new investments in research totalling $400 million since the government last January endorsed the international trade proposal that led to Bill C-91. The bill would extend market exclusivity for three years for most new drugs and modify Canada's system of 'compulsory licensing', which makes it relatively easy for generic drug companies to get permission to copy and sell brandname products.

The centre, to be located at the University of British Columbia (UBC), is seen as the first step in creating a fully fledged pharmaceutical industry on the country's western shore. Its director will be Michael Hayden, professor of medical genetics at UBC, who will investigate the way in which genes confer susceptibility to disease as well as trying to develop new treatments.

Hayden, who specializes in the genetic aspects of Huntington's disease, submitted a proposal for the centre to Merck, which has funded his research for several years. Hayden is director and a founder of the Canadian Genetic Diseases Network — a consortium of genetics researchers linked with eight universities and two industrial partners under the federal Centres of Excellence programme. One of the network's achievements was the recent codiscovery by Robert Korneluk of Ottawa of the gene that causes myotonic dystrophy.

The new centre will have major programmes in molecular genetics, in creating animal models for human genetic disease through gene targeting and transgenics and in gene therapy. Although the centre will be independent, its scientists will interact with Merck researchers worldwide and some will hold joint appointments at UBC.

"We will have a strong fellows programme to recruit postdocs at a critical stage in their career", said Hayden. "Individuals within the centre will be encouraged to raise support for their own research." Hayden expects to hire ten new faculty members in the first phase of a projected staff of 200 graduate students, postdoctoral researchers, visiting scientists and managers.

Merck's money will be used to operate the centre and support research, and Hayden expects the provincial government to pay for laboratories and office space. "We are hoping to have a commitment for the building by January and to occupy it by early 1994", he says. The programme will be affiliated with human genome projects in Canada and around the world, but its focus will be on biology, not technology.

The bulk of Canada's pharmaceutical industry is in Montreal and Toronto, with only one member of the Pharmaceutical Manufacturers Association of Canada, Quadrilogic, located in Vancouver despite efforts by the provincial government to attract other companies. Although approval of the bill is expected, opponents are concerned that its passage may lead to a sharp rise in the price of drugs. Provincial health ministers last month set up a task force to suggest ways to limit the extent to which companies would be allowed to raise prices.

**David Spurgeon**

# Toshiba to help US students think about technology

**Washington.** When the US National Science Teachers Association (NSTA) wanted to hold a group competition among US elementary and secondary students to stimulate their interest in how the world works, association officials knew it would not come cheaply. What NSTA needed was a high-technology company that saw the contest as a way to help strengthen US science education and to restore the country's sagging reputation for technical innovation.

Last week, the contest* was announced at a festive press conference featuring, among others, the actor who portrays Mr Sulu in "Star Trek". But the star of the show, the company with deep pockets and vision, was an actor more often cast as a technological enemy of the United States: the Toshiba Corporation, along with the Toshiba America Group Companies and the Toshiba America Foundation.

"American companies are too concerned about short-term profits to be interested in something of this magnitude", says William Aldridge, NSTA's executive director. "It's a shame. Lots of US companies are trying to help in the schools, but nobody is doing anything quite this big."

Toshiba has pledged $1 million a year, for at least the next three years, to finance a contest that NSTA expects to become an annual event. The contest, called Exploravision, asks students to imagine what an existing technology might look like in 20 years and how its evolution will affect society. Students must work in groups of four, and will compete for prizes in one of four categories corresponding to age.


Instead of the usual report, students must prepare a set of ten video 'storyboards' describing various points in their narrative. Those advancing to regional competition will be given money to make videos of their entries. The winning teams will be chosen next June in Washington, DC; first prize is a $10,000 US savings bond for each team member plus $1,000 in Toshiba products for the teacher and $6,000 for the school.

Unlike many programmes in which companies develop curricula and send trained personnel to neighbourhood schools, Toshiba will allow NSTA free rein to run the programme. "They are the ones who know what's needed, who to contact and how to do it", says John Sumansky, executive director of the Toshiba America Foundation.

Toshiba's corporate goals are equally straightforward. "We want to help children discover and articulate the role of science and technology in their lives", says Tadao Taguchi, head of Toshiba America and a director of the parent company.

Simple words, perhaps, but apparently they are still a bit too hard for a US-based company to utter. **Jeffrey Mervis**

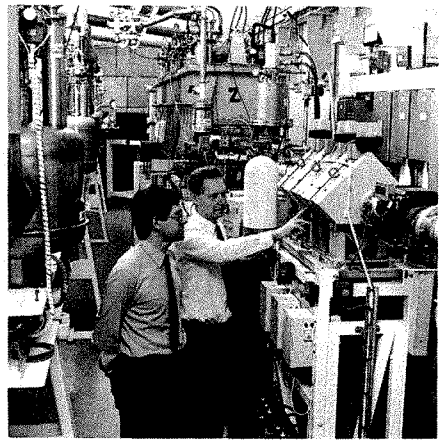
* For more information, write to NSTA at 1742 Connecticut Avenue NW, Washington, DC 20009 or telephone (202) 328-5800.

## Correction

The researchers at the US National Institutes of Health responsible for the first clinical trial of a gene therapy involving adenoside deaminase (ADA) deficiency were incorrectly identified in a recent article in *Nature* (**359**, 188; 1992). They are W. French Anderson and R. Michael Blaese. □

# UK nuclear physicists wonder if there is life after Daresbury

**London.** With Daresbury's Nuclear Structure Facility (NSF) closing in six months, British nuclear structure physicists are pinning their hopes on capturing a European physics centre expected to be built in five years. However, without an indigenous particle accelerator, their only chance of doing so lies in maintaining an active research community. That is why NSF officials are using equipment capital to buy beam time and set up collaborations around Europe and even in Australia.



**Linac is on the road again.**

The Science and Engineering Research Council (SERC) is studying a proposal on the feasibility of setting up such a European centre at ISIS, a pulsed neutron source based at its Rutherford Appleton Laboratory. But ISIS is not the only candidate for a centre to study intense (around 100 microamps) beams of radioactive particles; the GANIL heavy ion accelerator in Caen, France, is also expected to be a strong contender. Britain's success will depend on having a facility ready and on the vigour of its nuclear structure research community. Although the £1.5 million (US$2.7 million) test-bed proposal is expected to find favour with the SERC, its timetable of two years may be stretched to four because of a shortage of money.

In the meantime, the health of the British research community will depend on a band of 'scientific gypsies', working largely in collaborations at other facilities across the world. The most recent of these collaborations involves the donation of the Daresbury Linac (linear accelerator) to the department of physics of the Australian National University (ANU) at Canberra. In return, the British team will receive 20 per cent of the available beam time for five years.

The ANU accelerator was designed to accommodate a Linac-type device, but no funds were available. Although a European collaboration would have been more convenient for the British, the technical compatibility with the Australians made the deal very attractive.

The Linac has a chequered history. Originally purchased for the tandem accelerator at the University of Oxford, it had yet to be used when that facility was closed in 1986 in favour of the NSF. Transferred to Daresbury, it had been tested but never used experimentally when, in 1991, SERC made the decision to close NSF.

British researchers are concerned that their community will not flourish without a national facility. "Daresbury did us a lot of good and there was a great cross-fertilization of ideas," says John Sharpey-Schafer of the University of Liverpool. "A memory of what it was like will endure, but for how long?"

**Ian Mundell**

# British physicist to head CERN

**London.** Christopher Llewellyn-Smith, chairman of physics at the University of Oxford, is expected to replace Carlo Rubbia as director general of CERN, the European Laboratory for Particle Physics. The appointment, announced last week, should be confirmed at a meeting in December, and he will take up the position on 1 January 1994.

Llewellyn-Smith is chairman of CERN's scientific policy committee, and no major changes in programme direction are expected when the reins change hands. Within Britain, however, his appointment could strengthen the government's commitment to CERN at a time when currency fluctuations are robbing domestic research by increasing the cost of its international obligations. The Science and Engineering Research Council (SERC) estimates that the sterling crisis will increase by £10 million the cost of its European obligations.

Llewellyn-Smith was a vehement critic (see *Nature* **315**, 619; 1985) of the Kendrew report, which in 1985 recommended reducing Britain's contribution to CERN unless spending was trimmed. The report led to staff cuts and changes in the formula used to calculate each country's subscription. A SERC working party that reviewed the report earlier this year concluded that there was no cause to demand further alterations.

**Ian Mundell**

# Answers, but no solutions, for dispute at JET

**London.** British researchers at the Joint European Torus (JET) should be treated like workers at other European facilities, according to a new report to the European Parliament examining the bitter dispute between workers and management at the Culham Laboratory in Oxfordshire. But a question of jurisdiction threatens to delay for several months any resolution of the conflict, which has spawned several brief strikes at the experimental nuclear fusion project (see *Nature* **357**, 270; 1992).

The Budgets Committee of the European Parliament, meeting in Oxford last week, discussed a report by four independent consultants on terms and conditions at nine of the European Communities' joint research projects, including JET. The report made a number of recommendations that would resolve complaints by British researchers that they are being treated unfairly on pay and on employment prospects following the completion of the project in 1996.

The complaints stem from differences between the UK Atomic Energy Authority (UKAEA), which hosts JET and employs the British staff, and the European Atomic Energy Community (EURATOM), which employs all the other European researchers. The report's preferred solution is that UKAEA staff working on JET be offered temporary EURATOM contracts until the end of the JET project; the cost would be ECU7 million (US$9.1 million) at present prices. If that is not possible, the report suggests that UKAEA staff should be treated the same as EURATOM staff when researchers are hired for projects following JET.

However, the Budgets Committee refused to set aside the money on the grounds that the initial decision rested with the Energy Committee, which had commissioned the report. It said it would discuss the matter at its next meeting, on 7 October.

On top of this, the possibility of legislative changes means that the European Parliament's Legal Affairs Committee will have to be consulted. If and when the parliamentary committees give their support, the matter passes to the European Commission and, possibly, to the Council of Ministers.

Although British researchers at JET are pleased that the report vindicates their claims, they are no nearer to an improvement in their situation. Over the summer there were six strikes, each involving about a hundred people, and the union credits them with accelerating the report. A refitting of the torus is proceeding slowly, but JET management says that it is hard to tell how much of the delay is caused by the strikes and how much by routine technical difficulties.

**Ian Mundell**

# Congress prepares compromise plan to save Landsat, lower costs and improve service

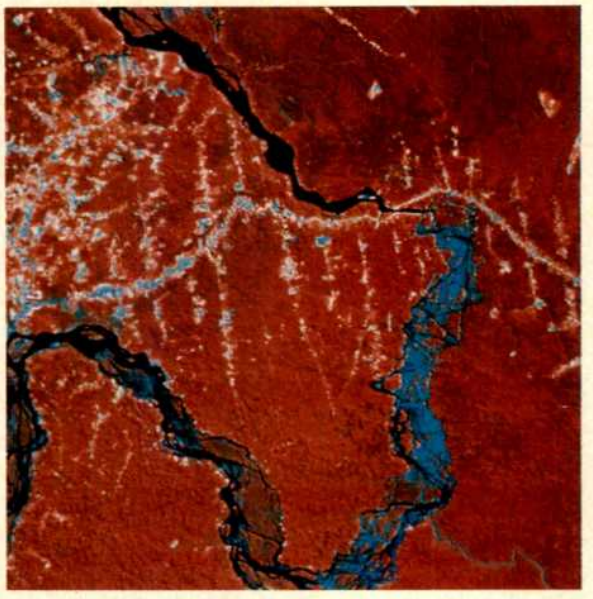
**Washington**. Landsat, the remote-sensing satellite programme whose move several years ago from the government to the private sector has been a case study in misguided privatization, may have won a new lease of life. A hard-fought compromise between federal agencies, environmental groups and Landsat's commercial operators is expected to return the programme's operations to the government, paving the way for a seventh orbiter and giving scientists renewed access to the data at minimal cost.

Early this week, Congress was putting the final touches to an agreement — a combination of a bill authorizing Landsat operations and data and appropriating money for a Landsat 7 orbiter in both the National Aeronautics and Space Administration (NASA) and Department of Defense (DOD) budgets for next year — that could be passed in the few remaining days before Congress adjourns. The last hurdle is approval from EOSAT, the company that now runs the Landsat programme.

If passed, the legislation would represent the end of the decade-long experiment in commercializing the Landsat programme. After years of losing money under government supervision, Landsat was transferred to EOSAT in 1986 as part of a series of such initiatives by President Ronald Reagan. But privatization was not the answer. Since 1982, when Reagan first tried to get the programme to pay for itself, prices have risen from $600 to more than $4,000 per image, and annual sales of images to the university research sector have dropped from 34,000 to just a few hundred. Landsat has also faced increased competition from the French SPOT satellites and projects by Japan and the European Space Agency.

The quality and availability of the data have suffered with the age of the Landsat 4 and 5 orbiters, launched in the early 1980s. Even those researchers who have been able to afford the images say that many of them are unreadable or are lost because they were recorded on used magnetic tape. Kevin Price, a University of Kansas geographer, rejected 13 of the 17 images he received last year. Others complain of waiting as long as four months for data and say that they often find it easier to hire a small aircraft to photograph the desired area. "Landsat's been nothing but heartache for us in the universities," says Price.

Users are particularly disturbed by EOSAT's policy of accumulating data only when there may be a market for them, making it difficult for users to purchase old data that they have not already asked EOSAT to acquire. Data-acquisition rates have dropped by 20 per cent (or more) a year for the past three years, leaving large gaps in the available data and making them almost useless for scientists who want to examine recent climate records or environmental trends.



**Landsat data — such as this Brazil deforestation image — may soon cost a fraction of their current price.**

EOSAT blames this situation on the loss of a Tracking and Data Relay Satellite in the 1986 explosion of the Challenger space shuttle, which left Landsat with only one relay satellite through which to send data, and officials say that it makes sense to collect data that it can sell. But critics say that it is another example of EOSAT's willingness to sacrifice science for profits.

Under the new agreement, the Landsat programme will be officially transferred from the National Oceanic and Atmospheric Administration to the DOD and NASA. A $20-million annual subsidy to EOSAT to pay for the operation of Landsat 4 and 5 — the only Landsat orbiters now aloft — will end, and researchers affiliated with the US global change research programme will be given access to data from Landsat 6, to be launched early next year, essentially at cost. Commercial users and those who have not signed an agreement not to use the data for commercial purposes would continue to pay a higher fee. Data from Landsat 7, to be launched in 1997, will be sold at cost to all, including commercial users.

Although EOSAT will have to make Landsat 6 data available at cost to many of its users, despite its investment in building and launching the orbiter, it sees the agreement as an opportunity to save the overall programme, protect Landsat 7 and remove continuing threats of cancellation. EOSAT hopes to win over users who have been unwilling to invest in special equipment to process Landsat data given the programme's uncertain future. And although it does not advertise the fact, EOSAT also stands to benefit from a commitment by Congress to build Landsat 7 because its corporate parent, General Electric, is the odds-on favourite to get the contract.

The current bill, a compromise between legislation introduced earlier by US Representative George Brown (Democrat, California) and Senator Larry Pressler (Republican, South Dakota), was expected to be voted on this week in the Senate. But because it requires unanimous consent, a single opponent could derail the process until next year. **Christopher Anderson**

# Canada apologizes to physicist

**Quebec**. The National Research Council (NRC) of Canada will not appeal against the decision of a tribunal of the Canadian Human Rights Commission that it discriminated against an Indian-born scientist, Chander Grover, on racial grounds (see *Nature* **359**, 95; 1992).

Complying with the tribunal's orders, it sent letters of apology to Grover and to the Optical Society of America, before which Grover had been forced to cancel an invitation to speak. A cheque for $5,000 and interest has been issued to Grover, and he has been appointed head of a 12-person optical components research group at the NRC.

The council says that its treatment of Grover was "not in any way based on race, colour or national origin but was the result of budget restrictions, shifts in research priorities and organizational restructuring". However, it has promised to review its policies and procedures relating to visible minority groups. **David Spurgeon**

# NAS to redo atomic studies found to be flawed

**Washington**. Two congressional agencies have found serious flaws in a 1985 National Academy of Sciences (NAS) study on the health of US servicemen exposed to radiation from atomic bomb tests. The study, which found a lower incidence of cancer in a group of 47,435 so-called 'atomic survivors' than in the general population, inadvertently included 4,500 servicemen who had not participated in the tests and omitted 15,000 who had, the investigations revealed.

In reports issued last month, both agencies — the General Accounting Office (GAO) and the Office of Technology Assessment (OTA) — blame the Defense Nuclear Agency (DNA) for sloppiness in assembling the data. Their reports, commissioned by Senator Alan Cranston (Democrat, California), the chairman of the Committee on Veterans Affairs, conclude that computer and transcription errors led the servicemen to be dropped from or mistakenly included in the agency's database. Although they do not suggest that the missing data would substantially change the 1985 report's conclusions, GAO and OTA argue that the study is important enough to do again, this time correctly.

The NAS, aware that the data were incomplete at the time of the study, has also come under fire for not acknowledging the fact. The only caveat was a footnote acknowledging that the readings on the radiation badges worn by many servicemen were not necessarily accurate and should be taken as approximate. The academy was under heavy pressure to complete the studies from veterans' groups, which wanted to confirm preliminary reports of damage, and from the military, which wanted to play down any medical consequences of the tests.

Robert Alvarez, a staff member for the Government Affairs Committee, which is investigating the atomic tests and related issues, says he is concerned that the flaws in the report may tarnish the academy's reputation for impartiality. He notes that the NAS was criticized in 1983 after it also found no excessive rates of cancer in a study of veterans who were in Nagasaki and Hiroshima after the atomic bombs were dropped.

In the 1983 report, the academy decided that the likelihood of a greater incidence of cancer from such low exposure to radiation was too small to justify a full epidemiological study. Instead, it attempted to contact 28 veterans who had claimed that they had developed melanomas. The National Association of Atomic Veterans denounced the report as "medically criminal" when it was revealed that the NAS researchers had discounted nearly half the cases because they could not confirm their location.

Seymour Jablon, one of the coordinators of both the 1983 and 1985 NAS reports and now a researcher at the US National Cancer Institute, recalls that the NAS researchers "had concerns from the very beginning" about the quality of the data in the 1985 study. "The Army data, in particular, were not in very good shape."

In 1979, the Centers for Disease Control found that veterans of one atomic test had twice the expected rate of leukaemia. Veterans' groups seeking medical compensation had hoped that a full study would confirm that link. The Department of Defense, on the other hand, did not believe that the low levels of exposure could cause lasting damage and hoped that the NAS study would disprove such a link. "There was an awful lot of pressure from both sides," Jablon says.

Another problem facing the academy is the fact that soldiers, because of their selection and training, are normally healthier than the general population. Some have questioned whether the academy took that bias sufficiently into account when it compared cancer deaths among survivors of atomic tests with statistics for US males of the same age.

Despite the flaws, Jablon believes that the study's basic conclusions are correct. Nevertheless, he argues that it should be redone with the new data, if for no other reason than to eliminate the lingering suspicion among veterans that the data were manipulated to avoid finding a cancer link.

In July, the academy agreed to do a $3.6 million, four-year follow-up study. It will attempt to avoid the healthy-soldier effect by using non-exposed servicemen as controls.

**Christopher Anderson**

# Japan endorses local touch

**Tokyo**. Local governments provide a growing share of public spending on research in Japan, according to the annual white paper (policy document) from the Science and Technology Agency (STA) released earlier this week (29 September).

The white paper is part of recent moves by Japanese science policy-makers to tap the resources of local governments, which in general are in better financial shape than the national government. Their mention in the agency's annual report is a sign that development of local regions is now an important part of national science policy and will no longer be left to initiatives from local governments or individual ministries.

The Council of Science and Technology, Japan's highest science policy-making body, which is chaired by the prime minister, earlier this year emphasized the promotion of science and technology in local regions as part of its call for a doubling of government spending on research. The white paper is a follow-up to that request.

The national government spends a little more than ¥2,000 billion (US$17 billion) a year on research, but that total has risen by only a few per cent a year because a national debt of ¥174,000 billion forces the government to put a rigid limit on spending. Local governments, on the other hand, supply a rising share of total government outlay on research — from 15.2 per cent in 1986 to 17.3 per cent in 1990, according to the white paper — and now spend close to ¥400 billion a year.

It remains true that the great majority of national research institutes, universities and private research institutes remain concentrated in the Kanto region around Tokyo. But some significant efforts to develop the science infrastructure in other local regions of Japan are under way.

The local governments and industry of Osaka, Kyoto and Nara are jointly developing the Kansai 'science city' in the mountains between these three cities. And although it may not deserve to be called a city, the Kansai development contains a significant number of new research institutes already open or nearly open.

Several initiatives have been launched in the past decade to strengthen local regions. MITI has tried to scatter giant science parks, called technopolises, around the country, but few have taken root. Two exceptions are those centred on Kumamoto in Kyushu and in Oita Prefecture, in the same southern island of Japan, which have used local and national government support to attract private and government research institutes.

Local government science policy has so far relied on the initiative of individual units, which has led at times to a duplication of effort. For example, nearly all local governments, regardless of their capabilities, have wanted to jump on the biotechnology bandwagon. To overcome this problem, the Council of Science and Technology has established a forum of high-level local and national government policy-makers.

Among other efforts, STA has formed a quasi-governmental organization to promote regional research, and next year MITI's Agency of Industrial Science and Technology plans to spend more on its regional research institutes outside of Tsukuba.

But each of these must compete with the magnetism of Tokyo. No matter what new institutes or science parks are opened, outlying regions may still find it hard to attract first-class researchers. **David Swinbanks**

# Gas leakage in United Kingdom

SIR — Independent analysts agree that gas leakage from cast-iron distribution systems is high. Half the gas distribution mains in the United Kingdom are made of cast-iron, pre-dating the 1969 change to natural gas, with a few dating from the Victorian era. Herbert's attack[1] on my Commentary[2] cannot hide this fact.

British Gas has been unable to refute figures in the 1990 study for Greenpeace[3] giving several per cent methane leakage; it has instead embarked on a measurement programme costing more than £2 million.

The company spends £40 million a year on adding 'conditioners' to combat the drying out and subsequent leaking of joints. But application is limited and inconsistent between regions. Droplets do not carry far and penetration to the periphery of the network was found inadequate. The data behind Greenpeace's 15 per cent effectiveness (low-pressure network average) is unrefuted. The new 'WISE' method involving direct injection into low pressure mains via standpipes has not been generally adopted.

British Gas keeps most figures secret. Herbert[1] objects to my use[2] of aggregated costs for pipe replacement that omit the customer payments, but not to my upper figures (obtained informally) which are only 50 per cent higher. Subtracting the expenditure on 'conditioning', at £300 per km (or £1,000 per km for low- or medium-pressure gas mains), the net annual costs of gas-pipe replacement (at 10 per cent discount rate) are £2,710 and £3,370, respectively, per km of mains.

At the median Greenpeace leakage rates[2], savings in lost gas at 20 p per therm are 33 per cent of the net discounted replacement costs, while at their upper leakage rates they are 67–99 per cent. Reduced need for inspection and repairs would add to these savings.

The above figures exclude the greenhouse pollution costs of the escaping gas. On the basis of the proposed EC carbon+energy tax (at $5+$5 per barrel of oil equivalent), I calculate 34 p per therm would be due on leaking methane. Using that notional figure, the value of gas saved by renewing median-leaky mains rises to 90 per cent of average discounted costs. Evidently, taxing methane pollution would provide a significant incentive to speed up the renewal programme (30 years to replace the cast-iron mains in the United Kingdom at current rates).

I suggest that the UK government needs first to repudiate officially the low leakage figure that British Gas has been unable to defend; second, to establish effective monitoring and controls on the methane pollution from fuel industries; and third, to consider the practicality of extending carbon/energy taxation to cover that methane pollution. Ofgas might be the appropriate regulatory body, rather than the Pollution Inspectorate (but see ref. 4), able to ensure that environmental impacts of gas leakage are covered in the tariff formula and/or performance standards.

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# Serbia defended

SIR – After a lengthy delay our library has finally received issues of *Nature* for 1991, where I found a letter, "Appeal from Croatia" (*Nature* **350**, 176; 1991), which requires a response.

For the third time in recent history, the Serbs are accused of resisting the new world order. In 1914, Serbs in Bosnia and Herzegovina did not want to accept their annexation by Austria. In 1941, Serbs began the war against Nazism by breaking the pact with the Germans, resulting in thousands of them being massacred by collaborators among the Croats and Muslim slaves. Now, this resistance is interpreted by the authors of the "appeal" as the ancestral sin of the Serbs, going back to the Middle Ages.

In support of the Croats' claim of a highly civilized origin, the authors of the "appeal" cite Nikola Tesla as a Croat scientist and Nobel prizewinner. This may be how Croats persuaded many other Nobel prizewinners to sign appeals against 'Serbian aggression' within disintegrated Yugoslavia. But a look at historical textbooks and biographies shows that Tesla was in fact a Serb from Krayina who never won a Nobel prize, even though his discoveries of alternating current, electromagnetic induction and the construction of the first power station were of immense value.

There is also much evidence that the Serbs have supported democracy in the past, for example the democratic movement of Serbian students in Belgrade in 1968 and the introduction of European democratic institutions in the Balkans after the Turkish occupation.

I wonder if readers took the trouble to check the facts in the "appeal from Croatia". The extent of anti-Serb propaganda throughout the world leaves colleagues and friends mute with astonishment. Serbian scientists and their colleagues are silently watching a new genocide of their people, hoping that the truth will somehow reach scientists elsewhere, who are supposed to be truth's servants.

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# Human insulin

SIR — Kiln and Sugarman[1] mention a number of diabetic patients whose awareness of hypoglycaemia was reduced during human insulin treatment but returned when they were transferred to porcine insulin. They claim that "no account has been taken of this fact by anybody". In fact, four separate studies have now been reported in diabetic patients selected specifically for having reported impaired awareness of hypoglycaemia during human, but not animal, insulin treatment[2–5].

Hypoglycaemia induced by human and porcine insulins was compared under laboratory[2,3] or 'free-range'[4] conditions, or both[5], in subjects who did not know which insulin species was being administered. None of these studies found any differences between human and porcine insulins in the physiological, endocrine or symptomatic responses to hypoglycaemia.

This issue has caused much concern among diabetic people and health-care professionals. Despite the weight of evidence that human insulin does not interfere with perception of hypoglycaemia, some diabetic patients may still have fears about human insulin and they should be transferred to animal insulins without delay and without argument. Otherwise, it is now time to turn attention to the many other pressing problems of diabetes. Kiln and Sugarman are worried that "we are still sitting on a human insulin timebomb". If they listen carefully, they may well discover that this particular timebomb has stopped ticking.

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# Coastal erosion and insurance

SIR — Barbara Culliton's leading article "Save the beaches, not the buildings" (*Nature* **357**, 535; 1992) is uninformed and self-contradictory.

Culliton writes ". . . the US Congress years ago agreed to provide flood insurance, at federal expense, for home owners whose beachfront property is damaged by storms and erosion". Congress did no such thing. It agreed to create an interest-earning fund to *underwrite* the risk to private insurers who provided such insurance. Congress has often chosen, in the public interest, to underwrite insurance that private insurers are reluctant to provide. An obvious example is bank-deposit insurance.

The distinction between underwriting insurance and providing it at federal expense might seem insignificant, except for the following. The federal government, and by extension the taxpayer, pays nothing for flood damage to private beachfront property. In other words, any claims for flood damage have been covered by the private insurers. It is worth adding that the overwhelming majority of the flood-damage claims are made not by the owners of beachfront property but by those of river-valley property. Hence the plaint that "taxpayers from the cornbelt [support] Eastern beach lovers" would seem to be the reverse of what actually happens.

Culliton inveighs against the efforts of beachfront communities, such as Ocean City, Maryland, to replenish their eroding beaches. There is no relation between such efforts and federally underwritten flood insurance. Where beachfront communities replenish beaches, they are doing so for the benefit not of beachfront home-owners but of members of the public who use the beaches for recreation.

As for self-contradiction, Culliton asserts that beaches are notoriously unstable and that it is folly for people to build on them. But if the beaches are unstable, what is meant by "Save the beaches, not the buildings"? As she objects to efforts to stabilize public beaches, what she has in mind for the beaches is presumably letting nature have its way. Whatever the wisdom of such a policy, it would seem to be an odd definition of saving. To be sure, there were those who felt that the great fires in Yellowstone National Park were a natural phenomenon that should be allowed to run its course. Few agreed with them.

On the whole, Culliton seems to hope that in time all beachfront homes will be bulldozed and the beaches will be left in their pristine condition. This is an arcadian vision unconnected with reality. Responsible beachfront home owners, living on a coastline extending from Maine to Florida, from Florida to Texas and from California to Washington, number in the hundreds of thousands. The notion that they can be made to disappear is no more reasonable than to expect that the entire country will one day revert to its pre-Columbian state. This being so, it hardly seems fair to deny citizens who live on the coast the right to insurance that costs the taxpayer nothing.

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SIR — Coastal erosion is not a simple universal continuing process at all. Where it occurs, it is usually the result of a combination of many processes, and a combination that changes significantly from place to place and site to site.

Hurricanes do tend to cause erosion, but not all of them. It depends upon their translational celerity, but again, for most of the time, the storm erosion is reversed during subsequent fine weather, and the beach returns. Then all beaches, as well as coastal barriers, are not so much "unstable" as mobile. Beaches absorb wave energy by allowing their sediments to move. Pressure is exerted against the waves by the weight of the sediments being moved through a distance. There is also much evidence available that suggests that the US eastern seaboard barrier islands are migrating, generally quite slowly, not because of wave erosion but from the natural sinking of the edges of the Atlantic plates and/or the dead weight of the vast volumes of sediments deposited on the continental shelf by major rivers.

There is also highly convincing evidence that much and often most of the US Atlantic and Texas coast barrier island erosion is manmade and certainly not the fault of "natural erosion" nor the coastal landowners who wish to live there. The major beach erosion in Florida has been caused by dredging, usually by the federal government, of barrier island crossings to deepen them for navigation. Until very recently, the dredged material, which originally came from the beaches anyway, was simply dumped offshore in deep water.

Similar evidence from Texas and southern California demonstrates that their beaches and islands are retreating because natural sand that once maintained the beaches, by being discharged onto them during floods, is trapped in state and federal constructed irrigation and water supply dams that cut off natural sediment supply. And the extreme erosion to Folly Island, South Carolina, is most probably the result of the trapping capacity of the great jetties leading to Charleston Harbor and their subsequent dredging, as in Florida. In fact, in Florida itself, well over half the crossings now being dredged are not natural, but manmade in the past by government bodies. The dredged jettied navigation entrance to Miami Port is still called Government Cut.

The selection of Ocean City, Maryland, to denigrate the value of beach nourishment is an example of slanting evidence by picking a worst case. There are many more cases where artificial nourishment, not just 'dumping', has been highly beneficial and cost effective. The major nourishments carried out in Pinellas, Broward and Dade Counties in Florida have been stunning successes, and repaired the damage done by earlier disposal offshore of dredged material. It is similarly notable that the dramatic sea-level rise of 3.5 metres in 20 years comes from an obsolete approximation arrived at by the US National Academy of Sciences in 1987. Current research — much of which has been reported in *Nature* — is now beginning to downgrade this type of estimate, and the climate models used are themselves still open to significant modification as more data become available. We simply cannot yet make any reliable predictions.

"Sensible coastal conservation" is much more than picking out coastal landowners as villains. Coastal conservation's first step must surely be to identify the causes of the erosion, and for much of the United States the causes may be found to be manmade, but manmade by others than the beachfront land-owners who want to live there. There may be sound reasons for reviewing coastal flooding insurance and the impact of FEMA, but let us have a balanced view of the whole problem of cause and effect first, before picking on the poor shorefront residents and making them the only scapegoats. Even seawalls need not, and often do not, destroy beaches unless there are other applied erosive forces also affecting the coast, and not all of them are natural.

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# Mark Kac

SIR — In "Energy levels by path integration" (*Nature* **358**, 707; 1992), for Kacs read Kac, *passim*. A singular mathematician indeed!

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# A mess of red pottage

*Max E. Tate and Dirk Enneking*

**The abundant *Vicia sativa* cultivar 'blanche fleur' from Australia is perceived by markets to be a cheap, protein-rich pulse, but is it suitable for human consumption?**

ESAU sold his birthright to Jacob for a mess of red pottage, a dish thought to be the red lentil (*Lens culinaris* Medik)[1]. Taking today's prices of US $800 per tonne, this particular pulse must seem to be an attractive crop to many farmers. Unfortunately, red lentils are a rather poor-yielding crop, so in the late 1980s, when it was noted in Australia that the pinkish cotyledons of the 'blanche fleur' cultivar of *Vicia sativa* L. bore a close resemblance to those of the red lentil after dehulling, it was but a short step to the creation of a brand new food export market.

Work in Europe in the 1920s (summarized by Barulina[2]) established the virtual omnipresence of *V. sativa* as mimics of lentil crops. Flowers were eliminated as soon as they appeared because they are a different colour from *L. culinaris*. Lentil mimics still occur: proteins characteristic of seeds of a lentil-like vetch (*V. sativa*) have recently been detected as minor foreign matter in Manitoba lentil crops[3]. A European lentil-like vetch (*Vicia* var. *lentil sperma*) was of sufficient concern for the International Organization for Standardization to propose test methods for its identification in 1989.

So an uninformed consumer may well be duped by lentil look-alike vetches. The somewhat spherical cotyledons of blanche fleur more closely resemble those of the common yellow split pea (*Pisum sativum*) than the flatter appearance of the lentil, but the colour (especially when coated with vegetable oil to enhance its attractiveness) bears a distinct resemblance to a paler form of red lentils. It is this mimicry of the red lentil which lies at the heart of the blanche fleur story.

The blanche fleur cultivar of *V. sativa* has been a useful stock feed and green manure crop in Australia since the early 1950s, growing readily in the mediterranean climate of southern Australia. But it is now perceived to be a valuable food-export cash crop for farmers: the latest export figures for the state of South Australia alone show that the export trade has grown from a mere 50 tonnes of blanche fleur in 1988–89 to more than 9,470 tonnes from July 1991 to early May 1992. Of this latter figure, 5,600 tonnes were sold as split red vetch ('red legumes' or 'red dhal'), which must be destined for human consumption (because the added cost of splitting (dehulling) would make it non-competitive in the stockfeed market). There are also significant exports from other Australian states, so that these figures are underestimates of the true magnitude of the trade.

There are at least nine reports in the literature of the toxic effects of feeding untreated *V. sativa* to pigs, mules, horses, ducks, monkeys, turkey poults and chickens. Of particular note is the important neurotoxin study of Ressler *et al.*[4]. These authors established that a 50% *V. sativa* diet fed to week-old chickens caused all the animals to die in less than one week; the observed toxicity could be quantitatively accounted for by the measured levels of L-$\beta$-cyanoalanine (0.1%) and its $\gamma$-L-glutamyl derivative (0.6%). In contrast to the acute toxicity in chickens, rats showed only a marked retardation in growth rate. So although there is considerable species variability, under no circumstances can untreated *V. sativa* be considered to be suitable for human consumption.

Cotyledon mimicry of red lentils (*Lens culinaris*, upper) by commercial samples of the 'blanche fleur' cultivar (*V. sativa*). Lower left, split red vetch; lower right, the same product coated with vegetable oil to enhance its visual appeal.

Analytical studies at the Australian Grain Academy in Victoria have established that the blanche fleur cultivar contains similar levels of $\beta$-cyanoalanine (0.1%) and perhaps higher levels (1.1%) of the $\gamma$-glutamyl derivative than those reported for *V. sativa* by Ressler *et al.*[4]. So unless it is detoxified before eating, the split red vetch exported from South Australia alone is sufficient to provide 56,000,000 × 100-g servings, each of which, before preparation, contains up to 1 g of neurotoxins.

Alarming though the neurotoxin content of *V. sativa* is, the fact remains that people do not eat raw legumes but process them by, for example, dehulling, soaking and cooking with or without straining at intermediate stages. The analytical data from Victoria suggest that up to 90% of these water-soluble toxins can be removed by the age-old detoxification practice of leaching, which involves soaking the dehulled vetch and discarding the washings. In a separate experiment, approximately 30–50% of the toxin was removed by cooking. Nevertheless, it is as well to point out that these cooking experiments measured the loss of the original toxins, not whether there was loss of biological toxicity *in toto*. In this respect, Harper and Arscott[5] established that extensive autoclaving (8 hours at 15 p.s.i.) of *V. sativa* was necessary substantially to reduce its toxicity to chickens.

Before the identification of the neurotoxin content, the sporadic use of white-seeded varieties of *V. sativa* as a pulse for human consumption has been reported[6,7] and P. Hanelt (IPK, Gatersleben; personal communication) suggests that its consumption is still extant in the province of Ratcha in West Georgia (CIS). Hegi[7] mentioned that 'sour lentil' (*V. sativa*) samples were confiscated by police in Berlin during the First World War and Danckwortt[8] tabulated *V. sativa* as a poisonous vetch. Nevertheless, there does not seem to be any mention in this period of any unequivocal case of human poisoning that could be attributed to *V. sativa*.

The United Nations Food and Agricultural Organisation has a food quality and consumer protection group based in Rome which was apparently unaware of the widespread sale or human consumption of blanche fleur when we contacted it. A member of the group wrote that its view was that in countries where pulses are eaten, most toxins would be lost during the common culinary practice of soaking and discarding the washings. To us, the important point seems to be

whether or not the blanche fleur is likely to be treated as if it were red lentils. Red lentils frequently have only sufficient water added to produce the final desired consistency, thereby retaining as many of the minerals and vitamins as possible. With a red lentil mimic such as blanche fleur this practice would inevitably result in higher consumption of neurotoxins. With the almost ubiquitous presence of water-soluble (and in many cases thermostable) toxins in nearly all legumes, leaching by soaking is easy to recommend and practice, but in many regions of the world water is a scarce and valuable commodity.

Thus it would seem that in practice there is very little international monitoring of new commodities supplied for human consumption and there is great reliance on self-regulation by exporting countries. Presumably this is based on the premise that if mistakes are made, it is the financial profit and credibility of the exporter which suffers most — but in this particular case, there seems to be much less concern for the health of the consumer.

## Lentils or vetch?

Is there any evidence that blanche fleur has been labelled as red lentils? In the past it has, as is documented in a letter to the *South Australian Stock Journal* dated 26 March 1992. The Victorian brokerage firm which negotiated the sales of the split red vetch to various buyers stated: "The first two shipments, a total of 400 tonnes were branded at the request of the overseas buyers as split red lentils, but described on the phytosanitary certificates as split vetch. All shipments since have been branded either split legumes or split red dhal."

It is obvious from this statement that not all the blame for this situation must rest with the exporter. Furthermore, the lack of background knowledge within this particular company is clearly evident in their comments in an earlier letter to the *Stock Journal*: "Your statement regarding neurotoxicity is without foundation. This is supported by the repeat orders we are now receiving from all our overseas buyers who have had almost three months to evaluate the quality of the product."

The department of primary industries and energy supervises AQIS, the Australian Quarantine Inspection Service, which issues the all-important phytosanitary certificate to exporters. It was a consequence of a request to us last year by a concerned South Australian exporter of blanche fleur that we commenced a qualitative paper-electrophoretic investigation, in which we clearly identified the presence of the unusual and characteristic blue ninhydrin spot of β-cyanoalanine in a sample of blanche fleur at a level similar to that in other *V. sativa* cultivars.

Much to our surprise, the exporter informed us in late in February this year that, contrary to our report of the presence of neurotoxins in blanche fleur, AQIS had issued a circular dated 11 February 1992 entitled "False Trade Description — Export of *Vicia sativa* Described as Lentils" in which it was stated: "Many varieties of vetch contain toxic amino acids such as β-cyanoalanine making them unsuitable for human consumption. The recently released variety Blanche Fleur is suitable for human consumption and in its split form has a similar colour, shape and size as lentil. However, consumer acceptance of this vetch as an alternative food source has been slow."

Our initial reaction was to be thankful for the common sense of consumers. But we then repeated and confirmed our earlier observations. The responsible minister was immediately notified of our concern on 25 February and again on 5 March, to which we received a reply on 25 March: "I am informed that you have raised your concerns with AQIS about the possible levels of β-cyanoalanine in the cultivar Blanche Fleur. I have also been informed that AQIS, in response to your facsimile, has informed the Grains Council, exporters and AQIS inspection staff that it is not possible to provide affirmation that the cultivar is fit for human consumption.

It will be a matter for the industry to establish to the satisfaction of competent authorities such as the National Food Authority that the levels of β-cyanoalanine in this or other cultivars render them fit for human consumption. AQIS would then take appropriate action on this advice."

Exporters have assured us that once a shipment leaves the wharf it can be sold and resold many times over, and hence the destination on the phytosanitary health certificate bears little relation to where it is eventually consumed. Nonetheless, the split blanche fleur from South Australia in 1991–92 was initially destined for Saudi Arabia, United Arab Emirates, Dubai, Oman, Jordan and Egypt. The whole vetch was consigned to Portugal, Italy, Spain, South Africa, Austria and the United States. Re-export from the Middle East to India, Pakistan, Sri Lanka and other countries is highly likely.

## What more can be done?

In the first instance, the consumers and the scientific community at large can be made aware that red vetch *V. sativa* is not the edible red lentil *L. culinaris* and that is the main purpose of this article. Second, the common practice of soaking pulses and discarding the washings substantially reduces the toxin level, and this should be encouraged. Third, cultivars with negligible neurotoxin content can be selected for by plant breeders, despite the probable decrease in yields that would result from insect or avian predation.

For those who wish to distinguish between these two look-alike pulses, the simple week-old chicken bioassay outlined by Ressler *et al.*[4] would provide an inexpensive alternative to either HPLC analysis or the Canadian protein electrophoretic procedure, and would thus be feasible for use in poorer countries. Even on the scale of the Australian exports, there have been no reports of acute toxicity among consumers. It is therefore probable that any untoward consequences have gone unrecognized or are close to symptomless. Nevertheless, Ressler *et al.*'s observations on growth retardation by feeding *V. sativa* to immature rats provide a warning that one particular potential usage, as part of a free midday meal for schoolchildren in Sri Lanka, which was briefly suggested in a market-research report for the Australian Grain Legumes Committee in 1991, should be forbidden. And the indirect introduction of blanche fleur into famine-relief programmes should also be proscribed.

In a protein-deficient world it is possible that suitably leached or genetically altered and thereby detoxified cultivars of *V. sativa* may come to be important as human food, just as other crops at first regarded as undesirable have been modified for human consumption either by post-harvest processing (soyabeans) or by genetic improvement (rape seed). Meanwhile, in the absence of any satisfactory data on the toxicology of β-cyanoalanine and its γ-glutamyl derivative, everyone should be on their guard for the appearance of deleterious effects from the rising human consumption of the blanche fleur cultivar of *V. sativa* marketed as 'red vetch', 'red legumes' or 'red dhal'. □

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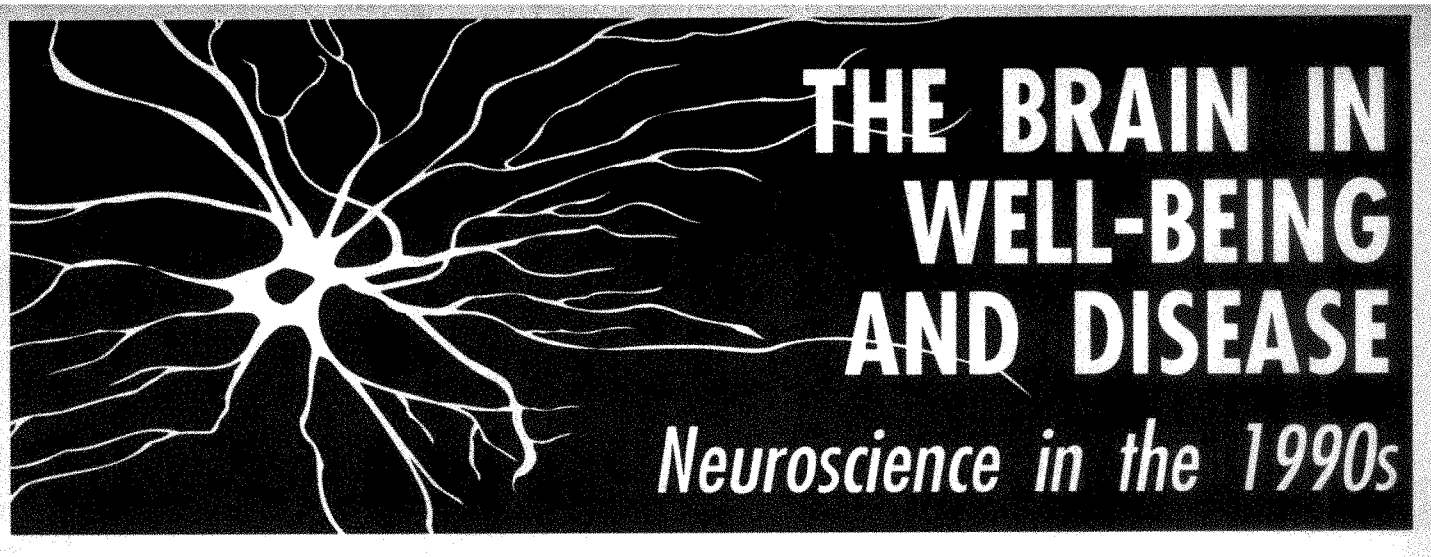


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# Forest fires, sandpiles and the like

**An elegant argument about the behaviour of a simple model of a forest fire has now been extended to make connections with other systems that keep their original organization in spite of external disturbances.**

On the principle that science is the art of the possible, in Medawar's phrase, the attribute of a physical model that matters most is that it should be tractable, not that it should rigorously embody what is known of the real world. Everybody knows that, for which reason the marvel is that so many models that incorporate only crude approximations to what is expected to happen on a microscopic scale nevertheless yield good or at least suggestive approximations to reality.

Forest fires are a case in point. Just over two years ago, Per Bak and Kan Chen from the Brookhaven National Laboratory and Chao Tang from the University of California, Santa Barbara, put forward a simple model of the process in which forest fires form (*Phys. Lett. A.* **147**, 297; 1990). It may be recalled that Bak and Tang (with K. Wiesenfeld) were the authors, in 1987, of the intriguing analysis of the stability of a sandpile in which they showed, among other things, that such a system displays what they called "self-organized criticality"; within reason, however sand is added to a growing pile, and whatever avalanches are as a consequence induced on its surface, the result will be a profile of constant shape determined only by the microscopic properties of the sand grains.

But how to model a forest fire? The starting point is a square lattice on a two-dimensional surface. At each lattice point, there may be a tree or a vacancy for a tree. Some trees may be on fire, others are still green. The evolution of the fire is then followed by means of successive finite steps in time (which makes the model a cellular automaton in the technical sense), and by using a few simple rules to get from one time step to the next. The basic rules are these: a burning tree will have disappeared by the next time step, but will have set light to all the trees that were originally its nearest neighbours.

If this were all, the model would be very dull. In a densely packed forest, a single burning tree would eventually set light to all the others, and the region would be left free of trees. But firebreaks extending the whole breadth of the region would confine the conflagration to one part of the forest only. But in a sparsely planted forest, isolated trees would remain alive after the fire had burned itself out elsewhere. But this model of reality is dull because it fails to allow for what happens in real forests — the emergence of new trees where there is room for them. Whence the third rule for the evolution of a forest, the assumption that at each time stage, there is a probability $p$ that a new tree will emerge at any vacant site.

Plainly, with these rules, if $p$ is sufficiently small, the forest fire will burn itself out (but firebreaks will not, on this occasion, leave some parts of the forest intact). What the authors were concerned to show is that when $p$ exceeds some critical value, the concentration of trees on fire will eventually reach a steady state in which, for example, it is possible to calculate (from numerical simulations) the number of trees on fire within a distance $r$ of a particular tree which is itself on fire. Nobody will be surprised that the outcome is a fractal. The number distribution increases with the radius $r$, but less quickly than $r^2$, the area involved.

For what it is worth, the model should serve well to represent not simply the burning of a forest but the spread of infectious diseases in a community. The original objective of the argument and the simulation that accompanies it was to throw some light on the phenomenon of turbulence, where the correlation of motions over distance is also fractal in nature. But the original paper is suggestive only.

The argument has now been carried further by B. Drossel and F. Schwabl from the Technical University at Munich (*Phys. Rev. Lett.* **69**, 1629; 1992), who argue that the behaviour of a burning forest is more complicated and more interesting when the probability, $p$, of the emergence of new trees is small and even tends to zero. Although Bak and his associates had concluded that, in these conditions, the correlation length of burning trees (or the average distance between one burning tree and all others in the same plight) would increase indefinitely to infinity, it has since been shown by numerical simulation that there emerges a spiral pattern of fire-fronts whose dimensions are of the order of $1/p$, the inverse of the growth rate of the forest.

Drossel and Schwabl even have a simple explanation of the phenomenon. When $p$ is small and if there is a steady state, most of the underlying forest lattice will have been emptied of trees. The result will be that clusters of new growth will emerge in the cleared ground and, by their isolation, will remain free from fire until the cluster to whch they belong has grown to merge with another in which there are burning trees. Accordingly, burning trees will invariably be included within large clusters which also include trees not yet set alight, and whose diameter will be of the order of $1/p$, whence the dimensions of the fire-fronts found in the numerical simulations. The system is not critical in the sense that the correlation length does not diverge more quickly than the controlling parameter.

So how to make the burning forest critical in this technical sense? Some way must obviously be found to ignite the trees that stand in isolated clusters. What better than lightning strikes, which may destroy intact trees by a random process like that which allows the emergence of new trees at cleared sites? Drossel and Schwabl show that, if the probability of a lightning strike at a particular site is $f$ per time step, the properties of the system as a whole are entirely determined by the ratio $f/p$.

The elegance of the continuation of this argument is that it derives most of the other properties of burning forests, made even more realistic by allowing that even burning trees do not disappear instantaneously, but that their demise is spread over several time steps, by simple dimensional arguments, which are confirmed by simulation. One of the conclusions, for example, is that the burning of a forest will correspond to self-organized critical behaviour if the time in which a cluster of trees burns down (of the order of the square root of $(f/p)$ in the two-dimensional case) is less than the growth rate of the trees (proportional to $(1/p)$), which must in turn be less than $(1/f)$, essentially the interval of time between successive lightning strikes at the same site.

It seems unlikely that these conclusions will be of immediate benefit to foresters and such people, although the parellels with the behaviour of real forest fires should be readily apparent in the way in which successive waves of fire seem to be entirely compatible with the maintenance of a forest in something like a steady state. The more interesting analogy is with the sandpile problem. What Drossel and Schwabl show for the forest case is that critical behaviour requires that the time for clusters of trees to burn down must be less than that required for them to grow individually. The sandpile analogue is that the slumping of an avalanche induced by the accumulation of material randomly is faster than the rate at which material is added to the growing pile. One of these days, it may even be possible to connect this argument with the urgent and practical business of turbulence.

**John Maddox**

PLANETARY FORMATION

# Finding circumstellar disks

*Stephen E. Strom*

THE possibility that many stars were once surrounded by the kind of circumstellar disk that once surrounded our Sun, and from which its present planetary system formed, is increased by new observations of newly born stars. Piirola *et al.*[1], on page 399 of this issue, and Menard and Bastien[2] have used high-resolution polarimetric observations of several young stars in stellar nurseries, and find such circumstellar disks in all cases.

Over the past five years, astronomers have assembled much evidence strongly suggesting that the Sun and other stars were built over periods ranging from 100 thousand to 10 million years by the accretion of material through circumstellar disks. Such disks are a natural consequence of the gravitational collapse of the dense rotating protostellar cores comprising molecular gas and dust from which stars form. Circumstellar disks are also the likely birthplaces of planetary systems. Towards the end of a disk's lifetime, when most of the mass contained within the disk has been accreted onto the star, microscopic dust is believed to agglomerate into larger grains and eventually into kilometre-sized planetesimals, which in turn are assembled into planets through inelastic collisions[3].

The tools are now in hand to make measurements which allow us to follow the evolution of circumstellar disks, from the moment of stellar conception to the time when planets form. The two stars studied by Piirola *et al.* in the constellation of Cassiopeia, V376 Cas and V633 Cas, are less than a million years old, have intermediate mass (2–3 solar masses, $M_\odot$), and are just emerging from the protostellar cores from which they formed. The presence of circumstellar disks is deduced in each case from the polarization pattern that results as starlight is scattered by micrometre-sized dust grains around the stars. Near-infrared (0.9 μm) polarized-light images of each star and its surroundings reveal a large region characterized by high polarization in which normals to the observed electric vectors lie along radius vectors drawn outwards from the central star; and a highly flattened region of very low polarization.

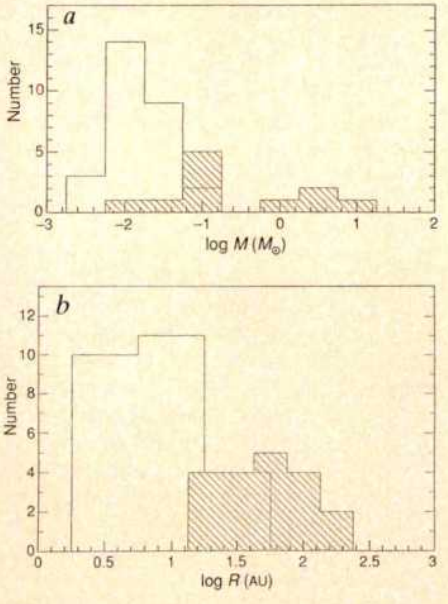


FIG. 1 Frequency distributions of (*a*) disk masses *M* derived from millimetre-wave measurements, and (*b*) disk radii *R* derived from infrared fluxes. Solar-type stars (mass between 0.2 and 1.5 $M_\odot$), blank histograms; intermediate-mass stars (over 1.5 $M_\odot$), hatched histograms.

The first pattern results from scattering of starlight by single dust grains located in low-density regions above and below the disk plane, and the second is produced as light propagates through dense regions near the disk midplane and is scattered by many dust grains before escaping, a process which dramatically reduces the magnitude of linear polarization. The larger of the disks, around V376 Cas, is 500–750 astronomical units (AU) in radius, making it 10–15 times larger than the Solar System. The smaller is too small to resolve. Menard and Bastien[2] have also used polarimetric imaging, in the optical part of the spectrum, to infer the presence of disks 100–1,000 AU in radius surrounding another 30 young intermediate- and solar-mass stars.

These polarization images provide the latest, most direct link in a chain of evidence indicating that young stars of all masses are surrounded by circumstellar disks. The most extensive evidence is indirect in character, from sensitive infrared and millimetre-wave photometric measurements of young stars spanning a range of masses. The presence of disks is inferred from radiation from heated dust grains distributed within the disk. At wavelengths much greater than 2 μm, the grains produce excess emission above the values normally expected for a 'diskless' star, giving a unique spectral signature.

A variety of basic parameters describing circumstellar disks can be inferred from the excess emission: millimetre continuum measurements[4,5] can give disk masses (Fig. 1*a*), and disk radii can be estimated from observed far-infrared fluxes[4] (Fig. 1*b*). Disks surrounding young stars apparently have radii comparable to or larger than the Solar System's and masses greater than the minimum necessary to build a planetary system (0.01 $M_\odot$). During early evolutionary phases, viscous coupling between the inner and outer disk transfers angular momentum out and drives mass in onto the star. From infrared spectra, we[4] have found mass accretion rates spanning from $10^{-8}$ to $10^{-4}$ $M_\odot$ yr$^{-1}$. By observing stars spanning a range of ages, we can also estimate disk lifetimes[6]. The infrared signatures indicative of accretion disks are most commonly seen around solar-type stars less than 3 million years old, whereas among intermediate-mass stars (3–10 $M_\odot$) accretion disks can be detected around only those younger than 0.3 million years. It is notable that the product of disk survival time and accretion rates requires that a large fraction of the material that goes into a star must pass through an accretion disk; apparently the disks are as important in building the central stars as in building any planetary systems.

FIG. 2 The edge-on disk of β-Pictoris, 400 AU across, as revealed by observations from Las Campanas. (R. Terrile, Jet Propulsion Laboratory.)

The only other directly imaged, well resolved circumstellar disk is that around the fully evolved star (over 100 million years old) β-Pictoris. This disk seems to be representative of the structures descended from the accretion disks around much younger stars. It was first identified through weak excess emission detected by IRAS, the Infrared Astronomical Satellite. As well as radiating long-wave radiation, the micrometre-size grains in the disk also scatter visible

radiation, revealing the edge-on disk directly (Fig. 2). The total mass of the grains is minuscule, less than the Earth's mass, but that there are any is surprising: radiation pressure from the central star will cause small dust grains to spiral onto the stellar surface within just 10,000-years, far short of the star's age (100–1,000 million years). The grains, therefore, must be replenished continuously.

The cratering record on our Moon records an epoch of heavy bombardment (within a billion years of formation of the Solar System), when collisions between planetesimals may have occurred frequently enough to create a steady-state population of small grains comparable to that seen around β-Pic. Backman and Paresce argue[7] from infrared data that β-Pic-like disks are quite common among stars in the solar neighbourhood, and that the assembly of planetesimals and even planets within disks may well be an inevitable consequence of disk evolution.

The next several years should witness significant progress in understanding the evolution of circumstellar disks. The European Space Agency's Infrared Space Observatory will allow quantitative estimates to be made of the total mass of micrometre grains in β-Pic-like disks surrounding stars anything from 1 to 1,000 million years old. In turn, these observations will allow estimates of the rate at which planetesimal collisions produce dust, and thus the frequency of such collisions during the epoch of planet formation.

Both large, single-dish millimetre-wave telescopes and more powerful millimetre-wave interferometers should soon provide the basis for statistical studies of the gas component of circumstellar disks around stars of different ages. Gas emission will provide kinematic evidence of disk material in keplerian orbit around the parent star, independent estimates of the mass of material contained within the disk, and a constraint on the time available for Jupiter-like planets to accumulate their massive gaseous envelopes. □

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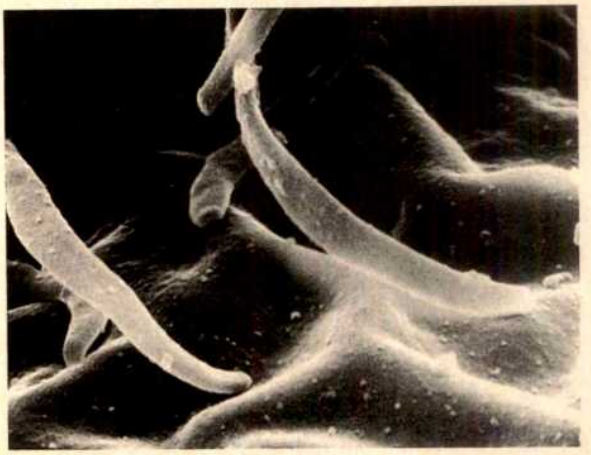
MALARIA

# Getting into the liver

*F. E. G. Cox*

THE protein that coats the surface of the infective stage, the sporozoite, of the malaria parasite has long been a molecule in need of a function. The ease with which this circumsporozoite protein has been characterized, cloned and expressed in a recombinant form has made it a favourite for vaccination studies, but with tantalizing results. Now, at last, its function is known, for Victor Nussenzweig and his colleagues have found that it acts as a ligand that binds to a receptor on the host hepatocyte[1]. This discovery answers a number of questions that have been puzzling malariologists, and it reinstates the circumsporozoite protein as a leading contender in attempts to formulate a vaccine against malaria.



Sporozoites, here seen escaping from an oocyst on the gut wall of the vector *Anopheles gambiae*. (From R. E. Sinden.)

Malaria infections begin when a mosquito injects sporozoites directly into a blood vessel. Very few sporozoites are actually injected, and they are carried around the body and are next seen in the liver hepatocytes. There they undergo a massive phase of multiplication to produce thousands of merozoites, which invade red blood cells and begin the destructive phase of the disease.

The spindle-shaped sporozoite, about 10 μm long and 1 μm in diameter, is coated with a single species of protein consisting of three main regions — an amino-terminal region containing the signal; the major component consisting mainly of multiple tandem repeats of four amino acids, asparagine, alanine, asparagine and proline (NANP in single-letter code); and a carboxy terminus containing an anchor sequence[2]. The repeat region has received a great deal of attention and has been incorporated in synthetic[3] and recombinant[4] forms into vaccines. Unfortunately, various vaccine trials have produced equivocal results, and the poor correlation between antibodies to the repeat region and protection against malaria in endemic areas[5] has forced scientists to look elsewhere on the molecule for alternative peptide sequences.

Of great interest are two regions, I, near the amino terminus, and II, between the repeat region and the anchor sequence. Region II has binding capacity[6] and is strikingly similar in sequence to the cell-adhesion domain of thrombospondin[7]; this is the molecule now incriminated in adhesion to the liver hepatocytes.

To understand this adhesion process, it is first necessary to say something about liver structure. The liver receives blood from both the hepatic artery and the hepatic portal vein, and this blood enters the sinusoids of the liver. The sinusoids are narrow channels, 10–30 μm in diameter and lined with a discontinuous layer of epithelial cells, and they communicate directly with the perisinusoidal space of Disse which borders the hepatocytes. The hepatocyte surface itself consists of short villi and about 70 per cent of the cell surface is in contact with the space of Disse. It is here that the transfer of materials from blood to hepatocytes and hepatocytes to blood takes place. Also lining the sinusoids are scattered phagocytic Kupffer cells derived from blood monocytes. How a malaria parasite enters a hepatocyte has been a mystery, some favouring the idea that entry into the cell is direct, and others (including myself) believing that the sporozoites are first taken out of the circulation by the Kupffer cells. It is now clear that they bind to and enter hepatocytes in the space of Disse, and that binding is accomplished by a region II sequence on the sporozoite.

The experiments concerned are elegant and leave no stone unturned. Using frozen sections of various tissues, Nussenzweig and colleagues[1] first showed that a recombinant protein, representing most of the circumsporozoite protein, bound only in the liver, and there only in the sinusoidal spaces. Other recombinant proteins containing regions I and II, but only one copy of NANP, also bound but those lacking region II did not; this region was therefore implicated in the binding, a conjecture which was

confirmed using a recombinant protein that represented region II. The actual binding site in the space of Disse was confirmed by immuno-electron microscopy, and binding to the hepatocyte membrane by homogenization of perfused fresh livers, density gradient centrifugation and immuno-electron microscopy.

From an experimental viewpoint, the use of whole livers is undesirable and it is important to know if region II is also involved in the binding of sporozoites to the widely used human hepatoma cell line, HepG2. Direct binding and inhibition experiments showed that this is indeed the case, an observation which opens up a much wider area of experimentation and provides an opportunity to investigate the effects of immunization with a synthetic peptide representing region II. Interestingly, the sera of immunized rabbits significantly inhibited invasion of hepatocytes yet reacted poorly with whole sporozoites, which might explain why anti-region II antibodies are so difficult to detect in the field.

These experiments show how malaria sporozoites get into hepatocytes directly, so there is no need to search for ways in which they evade the antimicrobial activities of Kupffer cells. The results can help to explain why there is a poor correlation between antisporozoite antibodies and protection, and — more importantly — they pinpoint a short sequence of amino acids that could be used as a target for immunization or chemotherapy. The circumsporozoite protein still has a lot going for it. □

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CANCER GENETICS

# Imprinting in leukaemia

*Wolf Reik*

GENOMIC imprinting — the parent-specific expression or repression of genes or chromosomes in offspring — is implicated in an ever-increasing number of diseases, not all of them rare or exotic. Examples range from uniparental disomy (in the Beckwith–Wiedemann syndrome) to preferential allele loss in recessive tumours (Wilms' tumour), and to preferential transmission of paternal or maternal predisposing alleles in diabetes or atopy (asthma and allergic rhinitis).

On page 414 of this issue[1], Haas *et al.* now add to this list what could be a major new category, namely reciprocal chromosome translocations in haematological malignancies. The new work shows that in the translocation leading to the formation of the Philadelphia chromosome, the hallmark of chronic myeloid leukaemia, the translocated chromosome 9 is preferentially of paternal descent whereas chromosome 22 is frequently of maternal origin. Because this translocation arises somatically during the life of the individual, genomic imprinting has to be involved either in the formation of the translocation or in its disease-specific selection.

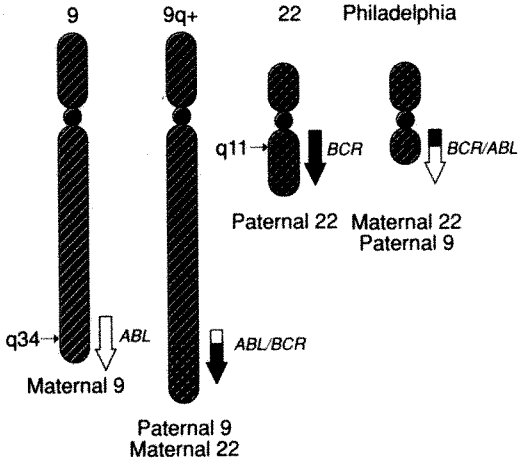
To determine parental origin of the translocated chromosomes in patients with Philadelphia-positive leukaemia, use is made of chromosome heteromorphisms. The differently sized centromeric heterochromatin on chromosome 9 and the differently staining nucleolar organizing region on the short arm of 22 provide polymorphic markers. Comparison of markers between the parents' chromosomes and the normal and translocated chromosomes in the patients thus allows one to assess the parental origin of the normal and the translocated chromosomes 9 and 22. Confusion could only arise if there were somatic recombination between the markers and the translocated regions, but I would assume the frequency of recombination to be too low for that to be of concern.

In 15 patients analysed, Haas *et al.* find paternal chromosomes 9 in the translocation in 11 cases, and maternal chromosomes 22 also in 11 cases. There is not a single case in which a maternal 9 or a paternal 22 is found to be involved in the translocation. It would have perhaps been mildly surprising if there were one nonrandom event, but that both chromosomes show a parental bias is quite unexpected. It suggests that two imprinting 'events' are involved, one concerning 9 and the other one 22, which may have important consequences for our understanding of how leukaemic disease arises as well as our concept of genomic imprinting.

How, though, does translocation between chromosomes 9 and 22 contribute to chronic myeloid leukaemia? Translocation at 9q34 and 22q11 leads to reciprocal fusion of the *BCR* gene (breakpoint cluster region on 9) with the *ABL* oncogene (homologue of Abelson leukaemia virus on 22). Potential fusion transcripts are produced on the Philadelphia chromosome between the 5′ portion of *BCR* and the 3′ part of *ABL* (*BCR/ABL*), and on $9q^+$ between *ABL* and *BCR* (*ABL/BCR*; see figure, and refs 2 and 3 for reviews). The BCR/ABL fusion protein has deregulated tyrosine kinase activity which has been attributed to the presence of first-exon *BCR* sequences[4], and the fusion gene can cause leukaemic disease when introduced into mice[2]. It is commonly assumed therefore that the little chromosome (the Philadelphia) is the culprit. The precise pathogenetic sequence of events leading to disease is not understood, however, although it is generally believed that the translocation is responsible for the early stages (expansion of the myeloid lineage) whereas additional genetic events are required for overt transformation (blast crisis). I am surprised at the lack of mention in the literature of the reciprocal product ABL/BCR (on $9q^+$), despite the recent identification of the BCR product as a GTPase-activating protein for the GTP-binding protein $p21^{rac}$ (related to the *RAS* oncogene, ref. 5). That the *ABL/BCR* fusion gene may also contribute to chronic myeloid leukaemia needs another look now.

Genomic imprinting can apparently be specific to a developmental stage as well as a tissue (for a summary see ref. 6). So far, at least three genes have been identified in the mouse as undergoing imprinting, and these genes have key functions in fetal growth and viability. At least one of them, *H19*, is also imprinted in the human. Importantly, imbalance of parental chromosomes and hence dosage differences of imprinted genes can lead to lineage-specific overproliferation of cells. In the mouse, malignant disease has so far not been observed as a consequence of altered dosage of imprinted genes.

The simplest explanation of the observation by Haas *et al.* is that both the *ABL* and the *BCR* genes are themselves imprinted. *BCR* would then be expressed on the maternal and repressed on the paternal chromosome 22, so that the *BCR/ABL* fusion gene is expressed following translocation of maternal but not paternal 22. Thus even if translocation were to occur randomly with respect to the two homologues, the maternal 22 translocation would be selected because of the proliferative advantage of certain stem cell compartments in which BCR/ABL exerts its effect. Conversely, the preferential involvement of paternal chromosome 9 suggests that the *ABL* gene may be expressed from the paternal but repressed on the maternal chromo-




Parental origin of chromosomes involved in the Philadelphia translocation. The typical karyotype of peripheral white blood cells from patients with chronic myeloid leukaemia is shown. Chromosomes 9 and 22 are translocated so that a normal chromosome 9 is present in addition to a $9q^+$ with some material from 22 attached to the long arm, and a normal 22 is seen together with the Philadelphia chromosome (22 with a bit of 9 attached). The translocation breakpoints are at 9q34 and at 22q11 and lead to the formation of a fusion gene between *ABL* and *BCR* on $9q^+$ and between *BCR* and *ABL* on the Philadelphia chromosome. The arrows indicate transcriptional orientation of the two genes. The study by Haas *et al.* shows that most translocated chromosomes 9 are paternal in origin (blue), whereas translocated chromosomes 22 are maternal (red).

some. This would lead to expression of *ABL/BCR* whenever the paternal 9 is translocated, in turn suggesting that both the BCR/ABL and the ABL/BCR products are necessary for leukaemia to occur. But this possibility seems to be at odds with gene-transfer studies in the mouse, in which *BCR/ABL* alone can induce leukaemic disease.

Could a look at the mouse be helpful, assuming that imprinting is conserved? The mouse *abl* gene is located on proximal chromosome 2, whereas *bcr* is on the middle part of chromosome 10. The *abl* gene, therefore, is in a region that is known to be imprinted (by genetic criteria), whereas the *bcr* region is not. Maternal disomy of proximal chromosome 2 leads to early embryonic death[7], and if the *abl* gene were responsible for this phenotype it should also be observed in mice with (null) mutations of their *abl* gene. Fortunately, the experiment has already been done (by homologous recombination), but the outcome is quite different from the prediction: mice without functional *abl* genes develop happily to term, whereupon they show reduced viability[8,9]. An effect of parental transmission of the mutant alleles has not been reported.

If *ABL* and *BCR* were reciprocally imprinted, we should expect to see this simply by looking at transcription of the four different RNAs in leukaemic cells of Philadelphia-positive patients. We should expect to see the *BCR/ABL* transcript but not the *BCR* transcript, and the *ABL/BCR* but not the *ABL* transcript. Again we draw a blank — leukaemic cells as well as leukaemic cell lines usually express messages for *BCR*, *ABL* and *BCR/ABL*. (Again, I find it curious that mention of *ABL/BCR* is not to be found in the literature, although presumably this fusion transcript could be of a size similar to *BCR/ABL*.) Imprinting of these genes, therefore, if present in some progenitor cell population, is not maintained in leukaemic cells that appear in the periphery. On the whole, however, it seems that there is little evidence in either man or mouse for imprinting of *ABL* or *BCR*.

There is another puzzling feature of transcription of *BCR* and *BCR/ABL*. When individual Philadelphia-positive haematopoietic colonies were analysed for expression, they were found to transcribe either the *BCR* or the *BCR/ABL* message, but not both at the same time[10]. Might random dosage compensation (as in X-chromosome inactivation) operate at a stage where parental imprints are no longer in place? Finally, if this were not enough speculation, it is possible that position effects are exerted by the translocation breakpoints on other imprinted genes on chromosomes 9 and 22. This could result in either an increased or a decreased dosage of gene products that may contribute to the proliferative potential of myeloid lineages.

The study by Haas *et al.* raises a number of fascinating questions, to none of which there is a simple answer at present. The Philadelphia chromosome still holds a secret or two. □

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RÉSUMÉ

## Relative differences

INTUITION if nothing else would tell one that moas and kiwis, both flightless birds (ratites) and both inhabitants of New Zealand, are especially close relatives. Not so, say A. Cooper *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **89**, 8741–8744; 1992). Cooper and colleagues applied sequence and phylogenetic analysis to part of the mitochondrial 12S ribosomal RNA gene of various ratites; moas are extinct, but the authors were able to extract usable samples from the tissue and bone of museum specimens. They find that moas diverged fairly early on in ratite evolution. Kiwis, by contrast, had a more recent ancestor in common with the emu, cassowaries and the ostrich. So New Zealand may well have been colonized independently by moa and kiwi forebears — with the possibility that the ancestors of kiwis arrived later by air, only then adapting to a ground-dwelling life.

## Acid test

PHOTOCHEMICAL reactions in cloud water droplets may be a significant source of organic peroxides and 'singlet' (excited-state) molecular oxygen, all key players in atmospheric chemistry (B. C. Faust and J. M. Allen *J. geophys. Res.* **97**, 12,913–12,926; 1992). The authors borrowed droplet samples collected by colleagues and irradiated them with simulated and natural sunlight. Although the resulting concentrations of oxidants seem small, a few nanomolar for the peroxides and less for the singlet oxygen under 'midday' conditions, they compete in importance with those generated by absorption into the droplets of the species created in air, previously thought to be the only process involved. The reaction mechanisms involved are not known. But given that peroxides and singlet oxygen are leading species responsible for oxidizing sulphur dioxide to sulphuric acid and for scavenging tropospheric ozone, the process clearly warrants further study.

## Smoke screen

A CAUTIONARY tale for the common man is told by G. D. Smith and colleagues, who after digging into a huge existing dataset have unearthed an association between smoking and suicide (*Lancet* **340**, 709–712; 1992). But this finding is one with a difference: although the association is strong, shows a relationship between dose and response, and seems clear of confounding factors, the authors simply don't believe it (or at least don't consider that here is a case of cause and effect). Their point is that epidemiologists are continually coming up with such associations, most of which may seem much more biologically plausible than their example but which may be equally spurious.

MELT DYNAMICS

# Convection brought to a focus

*David Loper*

ONE of the most remarkable forms of spontaneous self-organization in continuum mechanics is the formation of 'chimneys' during the solidification of liquid alloys (see figure). The nature and origin of these chimneys, focused columns of convection, have been studied for more than 20 years[1], but still remain poorly understood. On page 406 of this issue, Tait *et al.*[2] make a significant step towards a better understanding of this phenomenon by identifying the fluid flow pattern associated with the initial formation of chimneys.

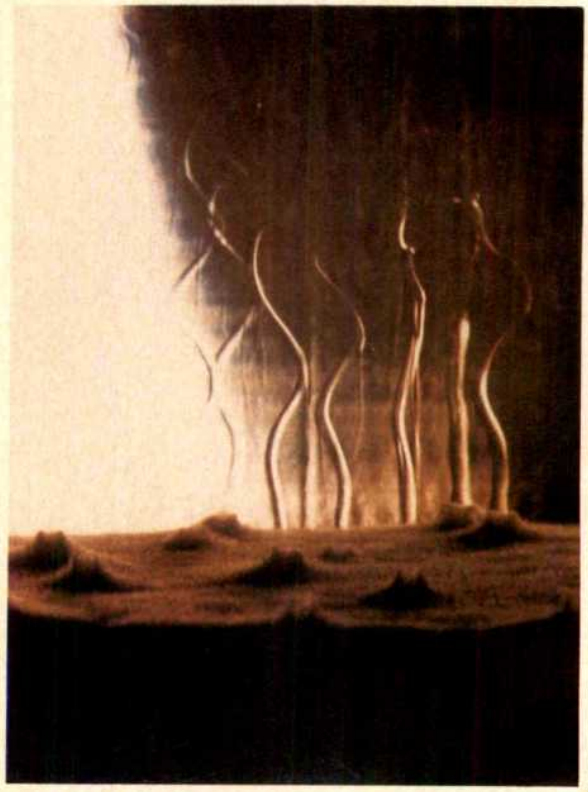
It is common to say that there are three phases of matter — solid, liquid and gas — but this is not the whole story. There are materials, such as plasmas, which do not fit this simple classification scheme. These materials have a microscopic structure which strongly affects the macroscopic dynamical behaviour. An important class of such materials is solid–liquid mixtures, composed of more than one constituent, in which change of phase occurs. These mixtures, which typically form when a liquid solution or alloy solidifies, are characterized by the mechanical configuration of the solid phase. One such is a 'mush', in which the solid forms a rigidly connected framework with liquid in the microscopic intercrystalline gaps. Mushes occur in various situations: during the casting of metallic alloys, in weld pools, the Earth's core and mantle, magma chambers, hydrothermal systems near mid-ocean ridges, temperate glaciers, frozen soils, frozen lakes and sea ice.

Chimneys in mushes were first studied by metallurgists in an effort to explain the occurrence of freckles which appear in unidirectionally solidified castings[1]. These freckles are the fossils of the chimneys, and significantly degrade the homogeneity and strength of the casting. Somewhat later, geophysicists realized that the situation identified by metallurgists is likely to occur in the core of the Earth[3], as well as in other geophysical settings[4]. The buoyant material flowing out of chimneys occurring in the solid inner core may induce the convective motions in the outer core which drive the dynamo that sustains the geomagnetic field; and the 'black smokers' (hydrothermal vents) observed on the ocean bottom near mid-ocean ridges are in effect chimneys in mushes. This interest in the dynamics of mushes is continuing; in the past six months, a metallurgical symposium was held in San Diego on the *Nature and Properties of Semi-solid Materials*[5], and a NATO Advanced Research Workshop on *Interactive Dynamics of Convection and Solidification* was held in Chamonix[6].

A mush is a new form of continuum, having novel physical properties not found in single-phase systems. For example, in a binary liquid system the density is commonly a linear function of both temperature and the concentration of the heavier component, with hot fluid tending to rise. However, if the system is constrained by the liquidus condition (determining the onset of the crystallization), temperature and composition are linearly related such that, for many systems, cold fluid tends to rise (see box).

In a mush cooled from below, the buoyant cold fluid emanates from solid-free chimneys which form spontaneously in the solid matrix. This process may be readily observed in the laboratory using an aqueous analogue of the metallic systems. The recipe is as follows. Dissolve 400 grams of $NH_4Cl$ in a litre of hot water, making a 28 weight per cent solution of ammonium chloride, place the container holding the solution on a bed of ice, and watch. Very quickly the bottom of the container will be carpeted with small dendritic crystals of ammonium chloride. These crystals grow slowly upwards into the liquid, looking very much like a forest of tiny fir trees, forming a 'mushy layer'. When the layer becomes about 2 centimetres thick, chimneys appear spontaneously within it. Buoyant fluid is readily observed to rise from these chimneys (see figure). The interdendritic fluid is compositionally buoyant because it has lost some of its denser component (ammonium chloride) during the solidification process.

The nature of the instability which leads to these chimneys is of particular interest both to practitioners and theoreticians, but has long been obscure. Theoretical analysis of the convective instability of a mush is hampered by the fact that the flow in the mush is coupled with that in the solid-free region above. The force balances for these two regions are fundamentally different and typically the fluid region above the mush is in a fully developed non-linear salt-finger convective regime.



Plumes of fresh water emanate from chimneys formed spontaneously in a mush of ammonium chloride crystals as a warm solution is cooled from below.

Before the new experiments by Tait *et al.*, it was not clear whether the instability leading to the formation of chimneys was intrinsic to the mush, or was driven by the salt-finger convective motions above. There were some indications, first noted at the recent NATO workshop, that the instability within the mush proceeds independently of the salt-finger convection. The study by Tait *et al.* appears to confirm that suggestion. They find the chimneys are the end result of a process which begins as a relatively large-scale hexagonal pattern of convective motion within the mush, with downward flow in the interiors and upward flow at the edges of the hexagonal cells.

The linearized equation of state for density, $\rho$, of a binary liquid system is

$$\rho = \rho_0[1-\alpha(T-T_0) + \beta(C-C_0)]$$

where $T$ is the temperature and $C$ is the concentration (mass fraction) of the heavier component. For normal fluids $\alpha>0$, so that hot fluid tends to rise. But if the system is constrained by the liquidus condition $T-T_0=\Gamma(C-C_0)$, the equation for density becomes

$$\rho = \rho_0[1-(\alpha-\beta/\Gamma)(T-T_0)]$$

For many systems $\beta>\alpha\Gamma$ so that, counterintuitively, cold fluid rises.

The dynamics of the mush are such that downward flow causes solidification and upward flow promotes dissolution. The upward flow is strongest at the corners of the hexagons, and the nonlinear interaction of dissolution and convection leads to the formation of chimneys at these corners.

The new insight provided by this experiment may be of use to metallurgists in their quest to suppress the occurrence of chimneys in solidifying castings. Also, this result may provide the basis of a theory relating chimney spacing to the depth of the mushy zone. Such a theory could help geophysicists constrain estimates of the number of chimneys occurring in the Earth's core, and provide a way to estimate the depth of the hydrothermal zone associated with black smokers. □

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QUANTUM MECHANICS

# Magnets get their act together

*Philip Stamp*

IT might seem remarkable that the study of magnets should still be throwing up novel ideas, but the evidence is there, in a remarkable new pot-pourri involving protein molecules from a horse's spleen[1], Feynman's work on 'quantum computers', and the old Schrödinger's cat paradox. What ties all this together is the idea that macroscopic magnets should behave entirely quantum-mechanically (that is by tunnelling or showing quantum interference between two macroscopically different states). If true, this idea could have profound implications in both fundamental physics and future technology.

Unfolding the saga requires a little knowledge of magnetism, a field of study older than science itself, dating to well before ancient Greek civilization. The quantum spins in each ion of a magnet interact with each other, principally via exchange interactions, so that in the magnetic state neighbouring spins line up parallel (for a ferromagnet), or antiparallel (for an antiferromagnet), in some direction (the 'magnetization vector' for a ferromagnet, and the 'Néel vector' for an antiferromagnet). Usually crystal fields make a few directions energetically preferable, often only two. On the macroscopic scale, it is apparently a matter of common experience that these vectors move classically.

## Belief

However, as first noted by Schrödinger, if we believe quantum mechanics, we need to explain why macroscopic systems behave classically. Moreover, as Caldeira and Leggett showed[2], in rare cases they may not — and there is now strong evidence for the tunnelling in large SQUIDs (superconducting quantum interference devices) between different flux states.

The situation in magnets may be even more remarkable. The modern story begins in 1986. First, in Grenoble, Barbara *et al.*[3] observed, for temperatures below 2 K, the sudden reversal of most of the spins in a large disordered ferromagnet (made of either DyNi or SmCuCo), and interpreted this as being initiated by the quantum tunnelling of the walls separating domains of different orientation, through the very large energy barriers created by the disorder. In the absence of tunnelling, the disorder should have rigidly pinned these walls, at these temperatures.

Quite independently, two theoretical groups[4,5] solved the mathematical problem of the tunnelling of a 'giant spin' (with spin quantum number $S >> 1$) through the energy barrier between two energetically favoured directions; this giant spin represents the sum of all the microscopic spins, aligned together along the magnetization axis.

The two tunnelling phenomena are not the same (one involves a giant spin, the other a 'giant wall', also containing many spins), and the tunnelling theory for a wall was worked out only later[6]. Nevertheless they both involve tunnelling between two macroscopically distinguishable states. This, however, was only the beginning of the story. The experiments of Barbara *et al.* were suggestive of tunnelling, but they did not prove it; and there was still the thorny question — why doesn't the dissipative or 'decohering' effect of the environment not completely suppress this tunnelling, as occurs for the overwhelming majority of macroscopic systems?

The latest experimental attack on these problems, by Awschalom *et al.*[1], involves the rather dramatic use of ferritin molecules, extracted from horse spleen. These proteins (relative molecular mass 480,000) have a giant antiferromagnetic ferrihydrite core, containing on average 4,500 $Fe^{3+}$ magnetic ions — in fact they are used by almost all eukaryotic cells to store iron. Awschalom *et al.* see a resonance in the electromagnetic absorption of a sample of ferritin molecules (diluted by a factor $10^3$), around 1 megahertz. This, they claim, corresponds to the promotion of the cores from their symmetric ground state ($\psi = (2^{-1/2}(|\uparrow> + |\downarrow>)$; $|\uparrow>$ and $|\downarrow>$ are the two Néel spin vectors) to their antisymmetric excited state, $\psi = 2^{-1/2}(|\uparrow> - |\downarrow>)$. Their claim is based on a qualitative agreement with a calculation[7] generalizing the giant-spin tunnelling calculations to antiferromagnetic grains.

If true, this would constitute the first ever observation of macroscopic quantum coherence (MQC) in physics — and this may be as close to Schrödinger's rhetorical cat as we will ever get. However it is necessary to be very careful here as such MQC is expected to be extremely difficult to see in any system — it is much more delicate[8] than tunnelling, involving phase coherence between repeated back and forth tunnelling events. Even non-dissipative couplings to the environment can destroy it.

## Verdict

So what about these environmental couplings? For straight tunnelling the theoretical verdict is, perhaps surprisingly, very favourable — for domain-wall tunnelling the dissipative couplings are very small[6,9] below 1 K, and walls containing $10^{11}$ spins or so should tunnel at millikelvin temperatures. Calculations for grains give similar results, at least for the coupling to phonons[10]. But DiVincenzo and colleagues (preprint) and I find that things may not be so easy for grains when it comes to MQC. Amusingly, only grains with integer spins can show MQC; if the spin is half-integral, MQC is completely suppressed, in line with Kramer's theorem. Unfortunately, at least for ferromagnetic grains, this probably renders MQC impossible, as the coupling to environmental spins will completely dephase the successive tunnelling events, leaving only incoherent tunnelling. For antiferromagnetic grains the verdict is still open, although doubts have been expressed here as well. (A. Garg, personal communication).

So why all the fuss? Well, even if it turns out that grains can't show MQC, domain walls are still viable candidates (they do not show this distinction between integer and half-integer spin). In

fact the experimental evidence for wall tunnelling continues to mount up (refs 11, 12 and D. D. Awschalom, personal communication), and there is good reason to suppose that tests of MQC on walls may be achievable in the laboratory. If so, a really very important test of quantum mechanics on the macroscopic scale may be within our grasp.

But there are other reasons for excitement. The use of biological molecules to do fundamental physics suggests we may be on the verge of a new kind of applied physics, involving biologically manufactured components. One of the more striking possibilities here involves a long-standing theorist's dream, the 'quantum computer', particularly in a form advocated by Richard Feynman during the last few years of his life[13]. In Feynman's model computer, all elements are two-state systems, set up to exchange and operate upon information in an entirely quantum-mechanical way — the information being encoded in the quantum state of the system. It is crucial that such computers are logically reversible. Moreover, if composed of giant spins such as those described above, they may show very small physical dissipation (at least, so says the theory). Recent unpublished simulations by J. C. Angles d'Auriac of the operation of such systems indicate they may be quite feasible, and if so this would have profound consequences for the next generation of computers. Back-of-the-envelope calculations indicate that a computing rate of around $10^{20}$ bits per second may be possible inside a hardware volume of 1 $cm^3$ which dissipates only 1 milliwatt at 0.25 K; this exceeds the present global computing power.

This of course is theoretical physics bordering on science fiction, and we must wait for experimentalists to catch up with theorists. But with such heady ideas in the air, it is small wonder that more and more experimentalists are accepting the challenge. □

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DEVELOPMENTAL BIOLOGY

# Big genes and little genes and deadlines for transcription

*Patrick H. O'Farrell*

SOME deadlines come and go, and the tardy are merely late. But some deadlines have a more exacting consequence, in which all tardy effort is futile. Transcription faces such a deadline — if a cell arrives at mitosis before RNA polymerase completes its sometimes lengthy task of transcribing a gene, then the nascent transcript is aborted. Like the mythical Sisyphus, RNA polymerase begins its task again, all to no avail, unless it meets the next mitotic deadline. From work reported last month by Rothe *et al.*[1], it now seems that the rapid mitotic cycles of an early *Drosophila* embryo impose deadlines that cannot be met by larger genes. The timing of these cell cycles is thus a biological 'gene screen' that precludes expression of large genes at early stages.

The synthesis of a transcript is a substantial task. The mammalian RNA polymerase working at its rate of about 3 kilobases (kb) per minute (37 °C) requires roughly 11 hours to traverse the gargantuan (2,000-kb) dystrophin gene. Measurements[2,3] of the lag between initiation at a promoter and transcription of downstream sequences have shown that the *Drosophila* polymerase progresses at about 1.4 kb per minute (25 °C). At this rate, 55 minutes are required for transcription of the *Ultrabithorax* gene, whereas only 2 are needed for the shorter *knirps* gene. Are these times of any significance to the function of the genes in the control of early development?

## Tempo

When copied by a queue of polymerases, the spacing between polymerases, not the total length of the gene, governs the rate of production of completed transcripts. So, although the length of a gene governs the lag time in appearance of completed transcripts, at steady state the yield of transcript is not influenced by gene length. But, in life, transcription is seldom a steady-state process. For example, the formation of pattern in the early *Drosophila* embryo is governed by a cascade of transcriptional regulation. It has been suggested that the lag times in the expression of different genes are important to the tempo of this cascade, and that evolution might tailor this parameter by adjusting intron length, and so gene length and transcription time[2,4].

Even when genes are not turning on and off, the staccato processes of the cell cycle disrupt 'steady state' transcription. Nascent *Ultrabithorax* transcripts are aborted as cells pass through mitosis[3], reappearance of completed transcripts in the next cell cycle being delayed by the 55 minutes required for the task. In cells that initiate transcription of *Ultrabithorax* too late in the cell cycle, the arrival of mitosis prevents completion of transcripts. These results revealed the deadline confronting transcription. Generalization to other transcripts would benefit from a second example, and the principle could be tested by reducing the size of gene that is too large to meet a mitotic deadline. That is what Rothe *et al.* have done.

The deadline for completion of transcripts arrives most swiftly during the rapid early cleavage cycles of the *Drosophila* embryo. These early mitoses are synchronous nuclear divisions in a syncytial cytoplasm. The first ten cycles occur every eight minutes, and of this eight minutes about five are occupied by events of mitosis. During cycles 11, 12, 13 and 14, the interphase period, and time allotted for transcription, lengthens progressively (4, 7, 16 and more than 65 minutes respectively)[5,6]. There is only limited transcription prior to cycle 14 (ref. 7), and the genes that are known to be expressed during these early cycles are predominantly small. One expects — and the paper by Rothe *et al.* confirms — that a large gene would not meet the transcriptional deadline imposed by these early rapid mitoses.

Rothe *et al.* examined a small gene and a big gene. The *knirps* gene is small (3 kb) and is classified as a gap gene: in its absence, a broad swath (gap) of the embryo is not segmented and fails to develop. The gene is expressed as both RNA and protein in cycle 13. Its expression is confined to a well-defined pattern within the syncytial embryo, and it functions locally as a transcription factor to guide striped expression of the subsequent tier of patterning genes. The *knirps* gene has a big brother, the 23-kb *knirps-related* gene[8]. They differ primarily in the length of their introns. The patterns of transcription and the protein products are similar, but still, *knirps-related* does not provide *knirps* function (ref. 1 and H. Jäckle, personal communication).

Although transcribed in cycle 13, the *knirps-related* RNA product does not leave the nucleus. The RNA product is detected with a 5′ probe but not a 3′ probe, so it is presumably an incomplete and hence nascent transcript. It seems to

be aborted at mitosis 13 without ever yielding a completed product. Cytoplasmic transcript finally appears about 20 minutes after mitosis 13, consistent with the expected transcription time of 16 minutes. To test whether this expression delay is due to transcript length, Rothe *et al.* trimmed the 23-kb *knirps-related* gene to 3 kb by removing the introns. This abridged version of *knirps-related* produces cytoplasmic transcripts and a protein product within cycle 13, confirming the adverse effect of gene length in a short cell cycle.

## Size

The abridged version of *knirps-related* partially complements a *knirps* mutation, demonstrating that the *knirps-related* product has a modest amount of *knirps* function. It is, however, unclear why the endogenous full-length *knirps-related* gene does not express this function. There are numerous differences between the endogenous gene and the cDNA transgene (note that transgene expression is driven by the 5′ regulatory region of *knirps* and the *knirps* promoter), but one of them — the reduced size of the transgene — points to an explanation that fits the molecular results to a T. As the authors argue, by precluding early expression of completed product, the size of the endogenous gene might prevent expression of *knirps* function.

A gene must be small to be expressed in short cell cycles, but why is *knirps-related* large? Although its involvement in head development occurs later, there is no obvious reason to preclude early expression of *knirps-related*. Because its transcription pattern roughly parallels that of *knirps*, early production of a completed *knirps-related* product should simply provide a redundant *knirps*-like function.

More generally, why should any gene be large, if size compromises function? There are many possible reasons — it might be important to preclude expression of large genes during rapid cell cycles, or delay expression in slower cell cycles, or introduce a temporal lag in a cascade of transcriptional control — but an example of any of them remains to be documented. □

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HUMAN GENOME PROJECT

# Mapping the way ahead

*Peter Little*

A REALISTIC solution to a long-standing problem in the Human Genome Project emerges this week with the publication of maps of most of two human chromosomes — chromosome 21, described on page 380 of this issue[1], and the Y chromosome, described in *Science*[2]. The Human Genome Project has a major mid-term goal, which is to construct a map of the whole human genome, and much debate has centred on the nature of this map. Should it be a physical map of restriction sites; should it be a recombination map; or should it be a map of cloned DNAs organized into overlapping arrays (so-called cloned DNA maps)? There are many models for all of these types of map. But opinion has been forming around the ideas that it should be a cloned DNA map and, because the genome is large, that yeast artificial chromosome vectors should be the cloning system; such vectors can carry up to 2 million base pairs of passenger DNA.

The two papers are the first indication that the debate has been resolved. What is exciting about them, apart from reporting the generation of the first such maps of human chromosomes, is that they both elegantly demonstrate that a reasonably simple approach can be applied to any genome without having to construct complicated libraries from individual chromosomes.

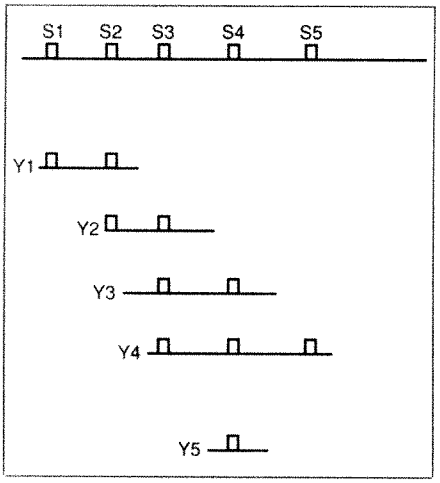
The work on the human Y chromosome, from the laboratory of David Page, and that on chromosome 21, from the consortium led by Daniel Cohen, both employ sequence tag sites[3] (STS) to generate the map. An STS is a short sequence of DNA that can be amplified using the polymerase chain reaction (PCR). STS have been positioned throughout the human (and many other) genomes and it is possible to carry out multiple PCR reactions with very little effort. The figure overleaf shows how STS can be used to construct a set of overlapping yeast artificial chromosome (YAC) clones that collectively represent

## Soft option for pigment analysis

**Q-TIPS or cotton buds (depending on where you come from) may come to the aid of art restorers and historians, if a technique developed by Luc. J. Moens, Wim J. Devos, Alex von Bohlen and Reinhold Klockenkämper, is taken up. Working at Ghent University and the Institute for Spectrochemistry, Dortmund, the researchers find that it is possible to analyse the few grains of pigment picked up by a clean, dry cotton bud wiped over the surface of a painting, and to get an elemental breakdown of their composition. The novelty is in the use of total reflection X-ray fluorescence, a technique which maximizes the efficiency with which X-rays excite the sample atoms to fluoresce. The simplicity of this benign method makes it an excellent alternative to the usual non-destructive (or *in situ*) techniques, which involve handling whole paintings. The workers have already used the approach in preparation for restoring several paintings, including *The Annunciation*, shown here, by the seventeenth-century Flemish master Van den Heuvel. Something like 'ultramarine sickness' has afflicted the painting, turning the deeper blue of folds in the cloak greenish. The analysis reveals the presence of smalt ($CoAs_2$) in the discoloured parts, which has probably been altered by moisture and carbon dioxide.** R. P.

the genome — this is the cloned DNA map. What Page's and Cohen's groups have done is to apply this on a grand scale.

The chromosome 21 project required 198 STS to be placed on over 70,000 YACs. To avoid the impossible task of carrying out 198 × 70,000 PCRs, the YACs were grouped into 92 pools. If a pool was positive, 28 sub-pools were screened that allowed the clone to be identified uniquely and the individual clone to be finally checked directly. In all, 810 YACs were isolated by this procedure, and they were then arranged into overlapping sets of clones by the process detailed in the figure. The STS were all located on the long arm of the almost acrocentric chromosome and cover some 40–50 megabases.




Identification of overlapping YACs using STS mapping. YACs Y1–5 are tested for STS markers S1–5, organized in the human genome as shown in the top line. Y1 and Y2 can be aligned because they both contain S2, Y2 and Y3 both contain S3, Y4 is positioned because it contains S3–5 and Y5 is placed because it only contains S4.

The Y chromosome analysis required 160 STS to be analysed on 10,368 YACs. The YACs were pooled into 18 groups: each positive pool was itself divided up into further sub-pools, allowing, on average, just 26 rounds of PCR to identify the YAC. This process allowed Foote *et al.*[2] to isolate 234 YACs which were then assembled into a complete map by STS alignment. The STS were all located in the euchromatic region of the Y and so the 30-megabase map does not contain the highly repetitive DNA and centromeric portions of the 60-megabase chromosome.

Despite the global efforts put into genome mapping, STS densities on both of these chromosomes were not high enough for the laboratories concerned to use published STS alone. Considerable time and energy had to be put into generating new STS markers: the chromosome 21 project required 85 new ones and Page's laboratory, as the group reports in a beautiful companion paper[4], required 104.

What is the use of the YAC map? Clearly, it has established a definitive STS map of two chromosomes but its primary role is as a resource for at least three areas of biology. Researchers using positional cloning approaches to isolate genes can go directly from flanking marker to YACs, eliminating the laborious, time-consuming (and often ineffective) approach of chromosome walking. DNA sequencers can now tackle whole chromosomes, opening up huge areas of study in evolutionary biology and the natural history of DNA sequences. Finally, the relationship of chromosome structure and DNA sequence can become technically addressable.

At the risk of belittling a substantial achievement, there are still some serious drawbacks to these YAC maps. Some 40% (chromosome 21) and not more than 50–60% (Y chromosome) of the YACs contains artefactual hybrids of 21 or Y DNA with DNA from some other chromosome. These 'chimaeric' clones are very problematic to work with — how do you know which piece of DNA comes from the correct genomic region? YACs are also generally awkward to isolate in pure form.

The *Caenorhabditis elegans* genome analysis project has always been a paradigm for the human genome project, and that team use cosmids (containing 40,000 base pairs of foreign DNA) as their primary resource. There is no doubt that, given the choice, the resource user would rather be given DNA fragments in a more tractable form than YACs. Cosmids are perhaps not the ideal cloning vector either, but it would be wrong to assume that YAC maps are the final solution to the problem; they clearly are not, but it is not so obvious as to what the next step should be.

That being said, it would be quite wrong to give the impression that these papers are not landmarks within the Human Genome Project. They represent a massive body of work and the important message is that it can be done and it is now only a matter of time (and money) before all human chromosomes are completed: we can be sure that the work will not stop at humans, and a full mouse map will be a marvellously powerful adjunct to the genetic analysis of this organism which is central to much of research in biology. □

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DAEDALUS

## Light fantastic

**ARTIFICIAL light is one of the few really important inventions. It is still imperfect. Fluorescent lamps flicker and have an odd, spiky colour spectrum; the tungsten-filament lamp runs so cool that most of its output is invisible infrared. Ideally, it should run at about 5,700 K, the temperature of the Sun's surface. It would then emit mainly in the visible spectrum, giving a clear, bright light indistinguishable from daylight.**

**The snag is that tungsten melts at 3,700 K, and begins to evaporate at a mere 2,800 K. The tungsten–halogen lamp cunningly counters this by running its filament in an atmosphere of volatile halogen. Evaporating tungsten atoms react with the halogen to form halide vapour. The halide molecules decompose again when they hit the hot filament, thus depositing the metal back in place. The old carbon-filament lamps were 'conditioned' in the same way, by running the filaments in an atmosphere of hydrocarbon vapour. Thin weak spots, with a higher local resistance, got hot enough to decompose the vapour, thus depositing carbon to thicken and reinforce the weak spot.**

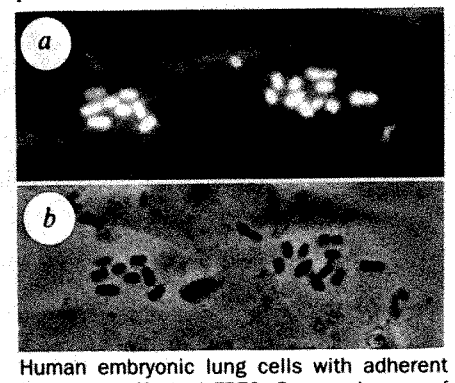
**In this connection, Daedalus recalls the new technique of chemical vapour deposition of diamond. A mixture of hydrogen and a hydrocarbon gas such as methane can deposit a thin coherent coating of diamond on a suitable heated substrate. Diamond has the highest melting point of any substance, probably greater than 5,000 K. So, says Daedalus, a tungsten or graphite filament, operated in a suitable hydrogen–hydrocarbon atmosphere, should rapidly acquire a diamond coating. It could then be run at up to 5,000 K or even higher. Encapsulated in diamond, the conductor could not evaporate even if it melted (tungsten would, graphite probably would not). And although the diamond would steadily evaporate, its hot vapour would react with the surrounding hydrogen to give a hydrocarbon which would deposit it back on the filament.**

**At a stroke, the humble light bulb will be transformed. It will give at least ten times as much light per watt, and that light will be pure natural sunlight. The harsh, head-aching fluorescent office environment, with its stroboscopic flicker and weird off-colours, will be banished. Indoor workers will regain that long lost instinctive outdoor cheer, and will even slowly acquire a slight but becoming natural tan. Indoor plants will flourish as never before. Daedalus was worried that a special ozone layer might be needed around the new bulbs to absorb damaging far ultraviolet, but he soon realized that the glass envelope could be designed to absorb that part of the solar spectrum.** David Jones

# Actin in cell attachment

SIR — In their News and Views article[1] discussing the potential of polycationic beads to model bacterial infection, Joseph and Jean Sanger ask whether the filaments found in intestinal cells infected with enteropathogenic *Escherichia coli* could be actin. The answer is definitely "yes". Since the first description of this effect[2], a considerable amount of work has clarified both the composition of this lesion and the mechanism by which it forms.

That the dense fibrous pad formed beneath enteropathogenic *E. coli* (EPEC) when they attach to various cell types is indeed predominantly actin was shown by staining with an actin-specific phallotoxin conjugated with fluorescein




Human embryonic lung cells with adherent 'attaching effacing' EPEC. Dense plaques of actin visualized by fluorescence staining with phalloidin (*a*) correspond exactly with each adherent bacterium seen by phase contrast (*b*). Magnification, approx. × 700.

(see figure). We have proposed this fluorescence actin staining test[3,4] for rapid detection of EPEC, which were previously identified simply on the basis of serotyping. With regard to mechanism, we have also demonstrated a localized elevation of intracellular free calcium in EPEC-infected cells of various types[5], and proposed that this activates the actin-severing function of cytoskeletal villin, leading to effacement of the brush border microvilli[6]. Subsequent dissipation of local increases in calcium concentration by natural sequestration into intracellular storage would then allow dissociation of villin, generating nucleation sites for an explosive burst of actin polymerization. This would deform the plasma membrane (now denuded of its microvilli) into the characteristic pedestal structure seen in natural disease.

As Sanger and Sanger point out, the possible involvement of nucleating proteins in this process is, as yet, unclear, but obviously we need to identify a bacterial potentiator of actin polymerization and the signal pathways in target cells. But our model does answer another question posed by Sanger and Sanger, this time in the negative. If, as they speculate, EPEC were able to cruise the brush border, destroying microvilli by mopping up the actin supply, we would expect to see regions of effacement devoid of bacteria. In fact, effacement is only ever seen at sites of bacterial attachment. A more likely explanation for gross effacement of microvilli is that EPEC infection is a dynamic process in which bacteria grow and attached microcolonies of bacteria continually enlarge, so that eventually microvilli are lost from the entire apical surface of an infected cell.

The EPEC pedestal also contains other cytoskeletal proteins in addition to actin[7,8]. Of particular interest is myosin, in which the light-chain component has undergone phosphorylation by the calcium-dependent enzyme myosin light-chain kinase[8], possibly as a result of a kinase cascade. Indeed, we believe that the success of EPEC as a pathogen resides in its ability to hijack the natural processes of cytoskeletal protein recruitment that are central to the healthy functioning of a cell.

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SANGER AND SANGER REPLY — The evidence of Knutton, Baldwin and Williams that filamentous actin is a major component of the dense fibrous pad localized in cells where EPEC attach is quite convincing. The electron micrographs of the fibrous material[4] also resemble images of cells in the process of phagocytosing bacteria or particles. It was the short columns of fibrous material extending into the cytoplasm beneath attached EPEC in other cells[2,9] that attracted our attention and reminded us of the columns of actin beneath the beads on *Aplysia*[10]. Assuming that these short columns are also made of actin, perhaps they represent an earlier stage in infection than that observed by Knutton *et al.*

As microvilli can reform rapidly under experimental conditions, it would be most interesting to observe EPEC on the surface of living cells. For example, Goligorsky *et al.*[11] have demonstrated that a one-minute exposure of cultured kidney cells to parathyroid hormone initially reduces the number of microvilli, but in five minutes the microvilli reform completely. Thus, bacteria might move on the surface of the host cell and microvilli could reform in their wake. On the other hand, Knutton *et al.* may be correct in proposing that attachment itself leads to effacement. The ability of bacteria[12] and beads[10] to "hijack. . . cytoskeletal protein recruitment" will enable systems to be devised that give valuable insight into both normal and pathogenic processes in cells.

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# Early nanotubes?

SIR — *Nature* has recently published several reports of the synthesis and structure of carbon 'nanotubes'[1–4]. The authors of these reports seem to consider the study of this form of carbon — a development since the discovery of Buckminsterfullerene[3]. But there was a report in *Nature* as long ago as 1953 by Davis *et al.*[5] describing very similar thread-like structures obtained from the reaction of CO and $Fe_3O_4$ and 450 °C on the surfaces of firebricks exhibiting 'iron spots'. The structures were described as layered threads, varying in thickness from 10 to 200 nm. The thicker strands appeared to be composed of many finer threads twisted into helical structures. The size and form of the threads appear to be similar, if not identical, to those described more recently[1–4].

As fullerenes are now known to be produced in sooting flames[6,7], it is not unreasonable to think that the structures produced by Davis et al. might have been nanotubes, although one certainly cannot tell this from their photograph.

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# Atmospheric cellular convection

SIR — The implication[1] that something might readily be deduced from the aspect ratio (width/vertical depth) of convection clouds observed on Venus, on the supposition that they are cellular in formation, is dubious. Laboratory versions of cellular convection, and most theoretical treatments thereof, are dominated by viscosity and thermal conductivity — that is, by molecular transfer mechanisms.

Clouds in the atmospheres of the Earth and Venus are under no similar influences. They are usually significantly affected by wind shear, which causes the cell size to be related to depth and makes them assume a particular orientation. When the wind is very light and without significant shear, as in the case illustrated in the figure, cells may continue to grow in size for a few days, especially if the heat gain and loss is from the sea and out to space by radiation from the lower and upper surfaces of the cloud and are thus dependent on no mechanical connection.

The depth of the cloud layer undergoes no significant change, and so in this case aspect ratios of 100 or more are achieved. There is no definite upper limit. If conditions are not fairly uniform over an area having a diameter of at least several cell widths, a regular pattern is not seen and definitions of cell size become variable and less obvious.

The apparent similarity of pattern in some atmospheric cases to laboratory examples of cellular convection is thus fortuitous; of more interest are the mechanisms of the upward heat flux by which the cloud is as often cooled as it is warmed.

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BAKER AND SCHUBERT REPLY — We agree with Scorer that the physical mechanisms responsible for convection in atmospheres and in the laboratory must be understood before significance can be attributed to similarities or differences in aspect ratios of convective structures. Precisely for this reason it is appropriate to investigate why the aspect ratios differ in the atmosphere and in the laboratory, or even among different atmospheres.

In the case of the atmosphere of Venus, we suggested a mechanism by which two convective layers interact to form a convective system that spans multiple scale heights and reduces the aspect ratio to typical values found in the Earth's atmosphere[1]. Although the dominant modes of forcing may differ between the two atmospheres (for example, release of latent heat for the Earth versus radiative absorption for Venus), the possibility that both atmospheres may exhibit convection with the same aspect ratio is attractive and should not be considered fortuitous.

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◀ This satellite picture (1246 GMT, 5 March 1982, Nimbus 7, 0.70–0.80 μm, minimum pixel size 0.8 km) of convection in a cloud layer about midway between Madeira and the Azores shows the gradual increase in cell size as the air moves slowly westwards and later northwards while the depth of the cloud layer remains almost unchanged and comes to a standstill in a pressure col. about 300 km from Santa Maria (the most southeasterly point of the Azores, marked S) and the cells become arranged in progressively larger units over possibly two or three days. This case is described in more detail in ref 2. (Copyright University of Dundee.)

# Allelic exclusion model questioned

SIR — Kitamura and Rajewsky[1] have presented evidence that targeted disruption of the membrane exon of the immunoglobulin class μ heavy chain results in a partial failure of allelic exclusion; that is, they have found another case of allelic inclusion[2,3]. Despite a rather poor agreement between their data and calculations, the authors claim that the 'feedback' of the membrane exon of the μ chain can account for allelic exclusion at the immunoglogulin H (IgH) locus and exclude the 'stochastic' model of Cohn and Langman[4]. We wish to point out that their results show a remarkable agreement with the predictions of a third model, the 'cellular selection' model.

This cellular selection model postulates that there is no feedback to stop rearrangement at the IgH locus, at least not until a functional immunoglobulin molecule with both heavy and light chains is formed, and that allelic exclusion at the IgH locus results principally from selection against cells producing cytoplasmic heavy chain from both alleles. The toxicity of free heavy chain[5] may be one factor, but by no means the only one, that could drive such cellular selection.

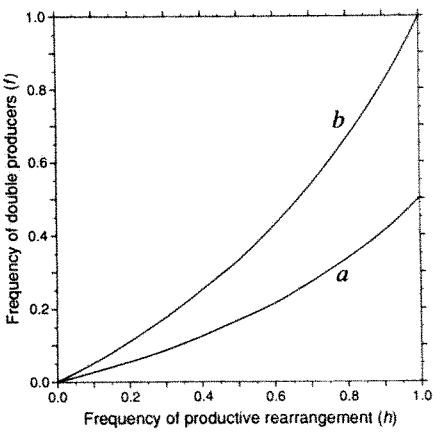
The cited results[1] were obtained with heterozygous mice in which only one IgH allele had a disrupted membrane exon; the other allele was wild type. Cells with allelic inclusion (namely cells that produce cytoplasmic IgH chains encoded by both alleles) were enumerated. The 'expected' values calculated[1], however, are not those that would be predicted using the authors' assumptions. Although this is not the principal reason for the lack of quantitative agreement, we present here the correct calculations.

Let $p_i$ be the probability that exactly $i$ chains are produced by a newly generated B cell. The frequency of double producers is $f = p_2/(1-p_0)$, because cells producing no IgH chains are not scored. With the feedback model of allelic exclusion, $p_0 = (1-h)^2$ and $p_2 = h^2/2$, where $h$ is the fraction of productive $VDJ$ joints ($V$, $D$ and $J$ are, respectively, variable, diversity and joining gene segments); the factor of 1/2 is needed because the mutant allele must rearrange first if both alleles are to be productively rearranged. Thus we get $f = h/(4-2h)$ (curve $a$ in the figure). With the cellular selection model, $p_0 = (1-h)^2$ and $p_2 = h^2$ (a factor of 1/2 is not needed because with this model it is irrelevant which allele rearranges first). Thus we get $f = h/(2-h)$ (curve $b$ in the figure).

With the feedback model, $f$ should be essentially the same for newly arising B cells in the bone marrow and for spleen

B cells derived from them. The cellular selection model predicts a frequency of double producers that is twice as great for newly arising B cells and a general, perhaps major, decrease in that frequency for spleen B cells.

Kitamura and Rejewsky present $f$ as 20–25 per cent for newly generated B cells in the bone marrow, which decreases to about 6 per cent in the spleen. For spleen cells the observed value of $f$ is compatible with both models. For newly generated B cells in the bone marrow, the feedback model predicts that $f$ should be less than 10 per cent if $h$ is less than 1/3; the cellular selection model predicts a value of about 20 per cent.




Expected frequency, $f$, of double producers as a function of the frequency, $h$, of productive *VDJ* rearrangements for two models of allelic exclusion. *a*, Feedback model; *b*, cellular selection model.

For technical reasons, a neomycin-resistance gene (*neo*$^r$) is inserted into the mutant allele; the authors discuss how that artifice might result in the values for bone marrow being too high. Without going into the validity of this point, we would like to point out that the decrease in the fraction of double producers cannot be explained in this way. The decrease in $f$ value from bone marrow to spleen is *ipso facto* cellular selection; this is a direct consequence of the undisputed fact that spleen B cells are the descendants of bone marrow B cells.

We are left with the difference between the knockout heterozygotes and normal, allotype heterozygotes. Unfortunately, no results were reported for normal, newly generated B cells[1]. For normal spleen B cells, $f = 0.3\%$, a value that ought to be different from 6% even though no statistics are given. If the authors are willing to contemplate that the inserted *neo*$^r$ gene might be responsible for the (to them) surprisingly high fraction of double producers in newly arisen bone marrow B cells, perhaps they should at least entertain the possibility that the *neo*$^r$ gene might also be responsible for the (to us) surprisingly high value in spleen B cells. Be that as it may, there is clearly a component of cellular selection against double producers.

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SIR — Kitamura and Rajewsky[1] described the effects of disrupting production of the normal membrane form of the immunoglobulin μ heavy chain ($\mu_m$) on allelic exclusion. We are concerned that readers of their paper might infer that it supports the concept of feedback inhibition, because many will equate the authors' conclusion that "$\mu_m$ signals allelic exclusion" with "$\mu_m$ stops heavy-chain rearrangement". We wish to point out that, although the authors have shown clearly that $\mu_m$ production affects the frequency of allelically excluded B cells, our understanding of the mechanism of allelic exclusion remains unsettled.

As outlined by Kitamura and Rajewsky[1], allelic exclusion in part reflects the *V(D)J* rearrangement process itself, in that the joining of the variable-region segments permits the loss of coded or addition of uncoded nucleotides, with the result that most rearrangements are expected to create out-of-frame joints. Were this the only contribution to allelic exclusion, one would still expect that about one-third (see below) of the immunoglobulin-producing cells would have two functional alleles, whereas in fact the frequency of mature double-producing cells is usually quoted as less than 1 per cent. Several explanations have been proposed to account for this low value. One type of explanation, that of feedback inhibition, invokes control of the rearrangement process, whereby the protein product of a functional rearrangement, such as $\mu_m$, immediately inhibits further rearrangement. Other explanations involve post-rearrangement effects, for example that cells producing a double dose of immunoglobulin are less viable, or that double producers which therefore bear surface immunoglobulin with non-uniform binding sites proliferate less after stimulation with specific antigen than do single producers.

The experimental system used by Kitamura and Rajewsky to detect feedback inhibition involved measuring the frequency of double-producing cells in mice heterozygous at the heavy-chain locus: the *a* allele was disrupted so that it could not yield $\mu_m$; the *b* allele was normal. Taking into account the possible stop codons in the *D* segments, the authors calculated the expected frequency of *a–b* double producers among IgM-positive cells as 12 per cent if $\mu_m$ inhibits rearrangement. To minimize the possible complications of post-rearrangement selection against double producers, they measured the fraction of double producers among newly generated B cells, reporting values of 20 and 25 per cent. These values were described as being greatly above the theoretical expectations, by which we suppose that the authors meant greatly above the expectations for a mouse in which a mechanism of feedback inhibition was operating.

They went on to propose an explanation for the high frequency of producers, namely that the disrupted allele was more prone to rearrangement than the normal allele, perhaps because it was rendered more accessible by the SV40neo transcription unit used to disrupt the $\mu_m$ gene; other explanations, some of which invoke artefacts of the culture system, are also possible.

This risk of artefact notwithstanding, we wish to point out that if there is no feedback inhibition of rearrangement, the expected frequency of double producers among IgM-positive cells is 24 per cent. The close agreement of this prediction with the reported values emphasizes the very interesting possibility that allelic exclusion arises because of post-rearrangement selection against double producers.

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RAJEWSKY *ET AL.* REPLY — We[1] did not mean to show "another case of allelic inclusion", as Wabl and Steinberg put it, but to test whether expression of the membrane-bound antibody heavy (H) chain of class μ inhibits further H-chain V-region (VH) gene rearrangement during B-cell development — a specific version of the 'feedback' model of allelic exclusion[2,3]. For this purpose, we used mice heterozygous for a null mutation in the membrane exon of the μ-chain. After polyclonal activation, B cells pro-

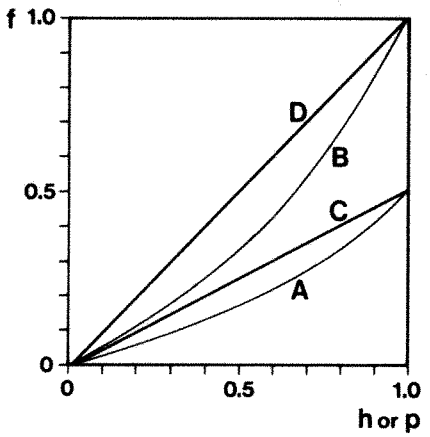


FIG. 1 Expected frequency, *f*, of double producers as a function of the frequency of productive VH→DHJH rearrangements in the feedback model (*h*) or the cellular selection model (*p*). For explanation see text.

ducing μ-chains from both chromosomes were indeed found in the heterozygous mutants, in contrast to the wild type[1].

Our critics say (1) that we calculated the "expected" frequency of double producers incorrectly; (2) that, in quantitative terms, our results can be better explained by the "cellular selection" model of allelic exclusion; and (3) that we did not look for double-producing cells in newly generated B cells of the control mice, where the cellular selection model would predict them to occur.

First, calculating the frequency of double producing cells in the heterozygous mutant mice on the basis of the feedback model, Wabl and Steinberg fail to take into account that cells expressing μ-chains only from the mutated locus disappear from the population because they are unable to express surface immunoglobulin[4]. Thus, all B cells in the heterozygous mutants carry (and express) a productive VHDHJH joint on the wild-type IgH locus. It follows that the frequency of double producers must be directly proportional to that of productive VH→DHJH rearrangement on the mutant IgH locus. This is borne out by Wabl and Steinberg's calculation after appropriate correction: the frequency of double producers is $f = p_2/(1-p_o-p_a)$ or $f = \frac{1}{2}h$, where $p_0$, $p_2$ and $h$ are as defined by Wabl and Steinberg, and $p_a$ is the probability of a cell to express a μ-chain only from the mutated allele, calculated as $p_a = h(1-h)$. The correct (linear) relationship between $f$ and $h$ as predicted by the feedback model is shown in our Fig. 1(C), with Wabl and Steinberg's calculation represented by curve A. At $h = 0.24$ (the estimated frequency of productive VH→DHJH rearrangement[5]) the expected frequency of double producers is 12%, the value we gave in our paper. If VH→DHJH rearrangements always start at the mutated locus in development (as discussed in ref. 1) $f = h$ and 24% double producers at $h = 0.24$.

Second, our critics claim that the 20–25% double producers in newly generated (unselected) B cells in the heterozygous mutant mice represent what the cellular selection model predicts. But in their calculation they disregard the finding[2] that most peripheral B cells of the mouse carry a DHJH, not a VHDHJH rearrangement on the nonproductive IgH locus, just as the feedback model predicts. Thus, although the units on the abscissa of Wabl and Steinberg's diagram correctly represent the frequency, $h$, of productive VH→DHJH rearrangement in the feedback model, in the cellular section model they represent the product, $p$, of the frequency, $t$, at which a VHDHJH joint is generated from a DHJH rearrangement and the frequency, $h$, at which this rearrangement is productive (Fig. 1). Assuming that after counterselection of double producers 60% of the B cells carry a DHJH joint on the nonproductive IgH locus[2], $p = 0.4h/(1-0.6h)$ or 0.11 for $h = 0.24$. At this frequency of productive VHDHJH joints the expected frequency of double producers is far below that seen in the heterozygous mutants[1] (D in Fig. 1 instead of Wabl and Steinberg's curve *b*; the calculation has again to take into account that cells expressing μ-chains only from the mutated chromosome are lost). Thus, the cellular selection model fails to explain the high frequency of double producers in newly generated B cells of heterozygous mutant mice.

Third, Wabl and Steinberg note that we saw some (0.3%) double producers in the spleen also of normal mice, claiming that we had not analysed newly generated cells in the bone marrow: 0.3% is not easily distinguishable from background staining in fluorescence microscopy. With regard to newly generated cells, we had analysed total bone marrow B cells from normal mice, about half of which represent newly generated cells[6]. We found less than 0.1% double producers on lipopolysaccharide activation. We have now directly analysed, by flow cytometry, newly generated B cells from the bone marrow of normal mice heterozygous for a μ-chain allotype for the presence of double-positive cells (Fig. 2). The frequency of such cells was 1.7% in the example given, and 0.7 or 2.1% in two other cases. These values are indistinguishable from background and probably represent a technical artefact. They are, in any case, substantially lower than predicted by the cellular selection model, and are radically different from those obtained in the heterozygous mutants[1].

Taken together, the experimental evidence weighs heavily against the cellular selection model, although selection

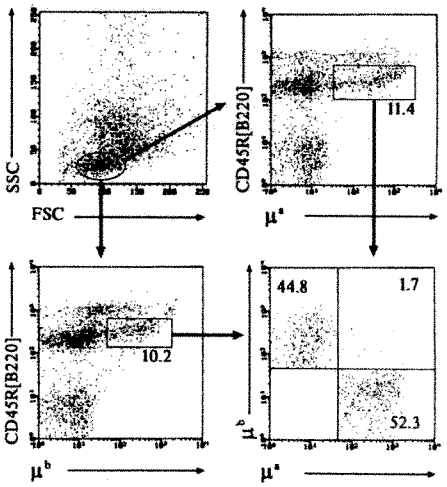


FIG. 2 Bone marrow cells of (C57BL/6×129)$F_1$ (IgH$^{a/b}$) mice were stained with fluorochrome-conjugated monoclonal antibodies, RS3.1–FITC (anti-μ$^a$), RA3–6B2–PE (anti CD45R/B220) and biotinylated MB86 (anti-μ$^b$) developed by streptavidin-Cychrome. Cells in the lymphocyte gate as defined by light scatters (upper left) were analysed on FACScan. Newly generated B cells defined as CD45R/B220$^{dull}$, μ$^+$ (μ$^{a+}$ 11.4%, μ$^{b+}$ 10.2%; upper right and lower left panels) were examined for single and double stained cells (lower right).

against cells expressing membrane-bound μ-chain from both chromosomes at an even earlier developmental stage (for example, that of the pre-B cell) does remain a formal possibility. We are in full agreement with Wabl and Steinberg's observation that our data demonstrate selection against double producers in the heterozygous mutant mice — that is why we decided to analyse newly generated in addition to splenic B cells[1]. But it seems that evolution has imprinted a mechanism of allelic exclusion into cellular development to prevent, in the normal mouse, the generation of those undesirable cells from the beginning.

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# The hydrological cycle and its influence on climate

Moustafa T. Chahine

**The uncertainties in assessing the effects of global-scale perturbations to the climate system are due primarily to an inadequate understanding of the hydrological cycle—the cycling of water in the oceans, atmosphere and biosphere. Overcoming this problem necessitates new ways of regarding a field traditionally divided amongst several disciplines, as well as new instrumentation and methods of data collection.**

THE hydrological cycle traces the largest movement of any substance on Earth. Water has always had, and will continue to have, a controlling influence on the Earth's evolution. Although the impact of human activities on climate cannot be assessed without including the role of water in all its phases, our quest is handicapped by the lack of quantitative knowledge about the distribution of water and by the need to understand its reciprocal interactions with the climate system.

The hydrological cycle influences climate in a variety of ways. The exchanges of moisture and heat between the atmosphere and the Earth's surface fundamentally affect the dynamics and thermodynamics of the climate system. In the forms of vapour, clouds, liquid, snow and ice, as well as during phase transitions, water plays opposing roles in heating and cooling the environment. Fifty per cent of surface cooling results from evaporation. Water vapour in the atmosphere acts as a powerful greenhouse gas and nearly doubles the effects of greenhouse warming caused by carbon dioxide, methane and all similar gases[1,2]. Clouds control climate by altering the Earth's radiation budget. The release of latent heat of condensation in clouds provides 30% of the thermal energy that drives the Earth's atmospheric circulation.

Given these diverse roles and the complexity of the exchange processes, we must treat the hydrological cycle as a whole, not in parts, to grasp its full behaviour and its intricate nonlinear feedback loops. This approach requires planning and coordination between theory, modelling and observations. It also requires the concerted efforts and expertise of people from many disciplines. At present a full 'systems view' of climate and the hydrological cycle has yet to emerge. This is due, in part, to the fragmented way in which the hydrological cycle has been studied, so that meteorologists, oceanographers, biologists and civil engineers all perceive it differently. The study and teaching of climate needs to integrate these disciplines.

Current efforts to improve our understanding of the hydrological cycle remain focused on observations and modelling. Coupling of land surface models with atmosphere and ocean models is a primary step towards climate prediction. To accomplish this coupling we face many conceptual and computational difficulties. Among them is the need to learn how to combine the dynamic effects of hydrological processes on different space and timescales in the presence of enormous natural heterogeneity.

Progress in many areas is limited by a dearth of accurate data. The Earth-observing satellite systems soon to be deployed by many nations will provide many of the necessary observations. Yet even these systems may fall short of providing some of the key observations not only of precipitation and atmospheric winds, but also of clouds, evaporation and ocean salinity. An international programme known as the Global Energy and Water Cycle Experiment (GEWEX)[3,4] is being implemented to observe and characterize the full hydrological cycle. This programme will endeavour to provide the essential remotely sensed and *in situ* measurements and will undertake modelling and field studies of the hydrological cycle.

My aim here is to provide an appraisal of our current theoretical and observational understanding of the roles of the hydrological cycle in the climate system, and its intimate connection to the energy cycle. I hope to show why the hydrological cycle has emerged as the central element in studies of climate change, and to anticipate the main advances expected in modelling and observations in the coming decade, along with areas where improvements will still be required.

## The hydrological cycle

Figure 1 shows the main known reservoirs and fluxes of water[5]. Not surprisingly, the oceans are the dominant reservoir in the global water cycle, holding over 97% of the world's water. In contrast, the atmosphere holds only 0.001%, and the rest is locked up in ice caps, snow and underground storage. The hydrological cycle is indeed global, because continents and oceans exchange water. Over the oceans, evaporation exceeds precipitation and the difference contributes to precipitation over land. Over land, 35% of the rainfall comes from marine evaporation driven by winds, and 65% comes from evaporation from the land. As precipitation exceeds evaporation over land, the excess must return to the oceans as runoff.

The mean residence time of water in the atmosphere and oceans is an important climate parameter. As Fig. 1 indicates, the atmosphere recycles its entire water content 33 times per year (total yearly precipitation divided by atmospheric storage),

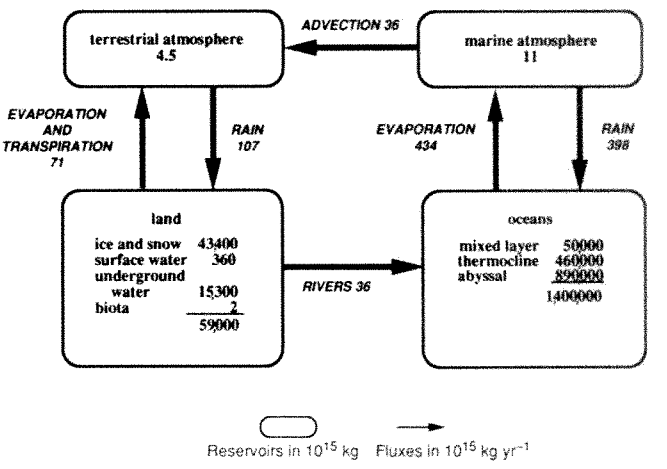


FIG. 1 Estimates of the global water cycle and its reservoirs. The accuracy of several of its components is poor, resulting in a closure error for the whole cycle of about a factor of two. The obvious interactive nature of the cycle makes it impossible to reduce current closure errors without studying the whole cycle. This diagram is based on Fig. 1 from ref. 5.

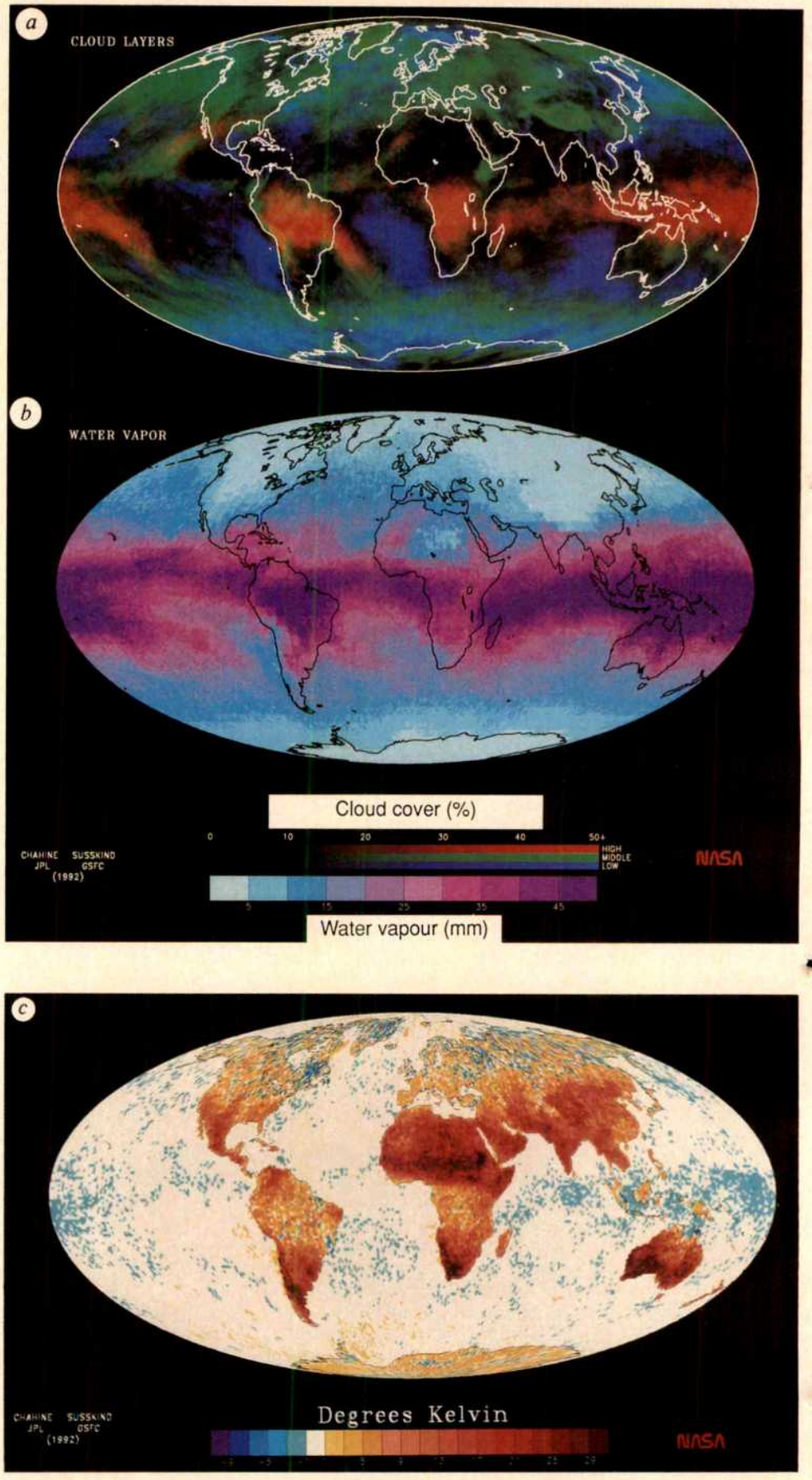


FIG. 2 *a*, Mean cloud cover and altitude for January 1979. Colours indicate cloud heights: blue for low clouds below 4 km (surface to 600 mbar), green for 4–8 km (600–300 mbar) and red for clouds above 8 km (300 mbar to tropopause). Shades of colours indicate variations in the infrared cloud amounts (opacity) as seen from satellites. The intertropical convergence zone (ITCZ) appears clearly as a region of a large amount of high (red) clouds that extends nearly continuously around the Equator. It shows several typical cloud streamers extending south-eastward into the western Pacific and northeast of Australia. In particular, the intense, high-level clouds above India, Indonesia, northern Australia and the western equatorial Pacific define the 'stratospheric fountain', where cold tropospheric air enters the stratosphere. *b*, Mean precipitable water vapour for January 1979. Most evaporation occurs in the tropics (dark purple) and is strongly driven by the amount of solar energy deposited at the surface. Comparison with *a* clearly shows the strong correlation between the distributions of water vapour and clouds. Among the driest regions on Earth are the Tibetan Plateau and Antarctica (light blue), which always appear dry in all seasons. The satellite data were acquired by the High Resolution Infrared Sounder (HRIS) and Microwave Sounding Unit (MSU), both instruments flying on-board the National Oceanic and Atmospheric Administration (NOAA) weather satellites. *c*, Inhomogeneity of land surfaces is clear in this image of temperature difference for day minus night, observed at 2 p.m. and 2 a.m. local time, for January 1979. A striking contrast exists between oceans (mostly white, denoting differences in the range ±1 K) and continental areas. In fact, one can sketch the contours of all continents from this image simply on the basis of the inhomogeneity of the day/night temperature difference of the land surface.

**BOX 1 The Global Energy and Water Cycle Experiment (GEWEX)**

GEWEX, one of the world's largest geoscience experiments, was initiated in 1988 by the World Climate Research Programme (WCRP) to observe and model the hydrological cycle and energy fluxes in the atmosphere, at the land surface and in the upper oceans. The ultimate goal of the programme is to acquire the necessary knowledge to predict variations of the global hydrological regimes as well as changes in regional hydrological processes and water resources.

Because of the magnitude of the effort, GEWEX will collate information from several ongoing process studies and will initiate investigations of its own, as needed, to improve modelling accuracy and the surface–atmosphere coupling in general circulation models. A build-up phase from 1993 to 1998 (or later) will be followed by a global observing phase coinciding with the deployment (around 1998) of global Earth-observing systems by the world's space agencies. The central activity of the build-up phase will be the GEWEX continental-scale international project (GCIP) and process studies designed to provide the parametric formulation of clouds, radiation and surface forcing factors.

The goals of GCIP are to determine the variability of the hydrological and energy budgets over a continental scale, to develop and validate coupled atmosphere–surface hydrological models and to provide a basis for translating the effects of future climate change into impacts on water resources on a regional basis. GCIP will be located in the Mississippi, one of the world's largest river basins. The effort will collect wind and rain data at high temporal and spatial resolution, satellite cloud and radiance data, cloud properties and radiation fluxes and other relevant hydrological data. GCIP will cover several annual cycles of hydrological processes; the overlapping period between GCIP and the global space-based observations from 1998 to 2000 will serve to validate information retrieval schemes for the new space observations and provide first-hand understanding of the relationship between direct *in situ* data and the data retrieved from space.

giving water vapour a mean residence time in the atmosphere of about 10 days. In contrast, the mean residence time for the oceans as a whole is over 3,000 years, but it is not the same at all ocean depths. In the ocean surface layers, it is only a few days or weeks, increasing to centuries and longer for the deep ocean levels. Over land, the mean residence time of water in vegetation and soil and in aquifers is difficult to determine but ranges from 6 years for the former to 10,000 years for the latter[6].

These two domains of residence time, days-to-weeks and decades-to-centuries, control the Earth's climate system in two distinct ways. The fast regime, consisting of the atmosphere, upper ocean layers and land surface, determines the amplitude and regional patterns of climate change. The slow regime, consisting of the bulk of the ocean, land glaciers and ice caps, modulates the transient responses of the climate system and introduces considerable delay. The fast component of the hydrological cycle has a critical role in predicting climate change and is the primary focus of GEWEX (see Box 1).

## Modelling the hydrological cycle

A continuous exchange of water among the reservoirs shown in Fig. 1 occurs mainly through evaporation and precipitation. The driver for this exchange is the Sun's differential heating, which varies with latitude, but the exchange pathways are controlled by surface properties and atmospheric and ocean circulation. Energy and hydrology in the climate system cannot be modelled separately, as they are linked by many atmospheric and surface processes. When an energy imbalance occurs in the atmosphere or at the surface, the atmosphere–surface system reacts to re-establish the balance. In the atmosphere, balance is most efficiently re-established by means of transport of latent heat through evaporation and condensation.

The basic tools for studying these exchanges are general circulation models (GCMs), which have been developed to depict the behaviour of the atmosphere. Over the past decade atmospheric GCMs have improved substantially and are now valuable for forecasting weather up to 10 days. At present, however, this level of confidence does not extend to climate forecasts. Climate models must still be developed to account for the full hydrological cycle and its interactions with the atmosphere, oceans and land. Considerable difficulty arises because the hydrological processes that must be integrated are nonlinear and function over widely different scales in space and time.

Scaling of nonlinear behaviour is a difficult problem. For example, $x$ cm of precipitation falling on 10% of a model grid square may produce a very different response from $0.1x$ cm uniformly falling over the entire square. The issue of the quantitative relationships between identical processes occurring at different scales of space and time is commonly encountered in calculating the fluxes of moisture and heat from a microscale ($1 \times 1\ km^2$) land surface model to a GCM with a grid size of $100 \times 100\ km^2$. Similar problems are encountered when inferring subgrid properties from the output of a GCM.

When atmospheric and surface models are coupled, they should be able to interact with each other dynamically at the interface. Achieving this will require advances on three fronts. First, we must improve our understanding of the physics, chemistry and dynamics of processes themselves, which requires accurate observations and carefully designed field experiments. Second, we must resolve the problem of representing great natural inhomogeneity; this fundamental statistical-dynamical problem remains basically unsolved. Third, we must translate the results of process studies to the global scale. These advances will be aided by growth in computer power allowing the use of climate models with smaller grid sizes. The task is enormous, but tangible progress is expected over the next decade.

## Interactive hydrological processes

I will focus here on five main components of the hydrological cycle: clouds and radiation, atmospheric moisture, precipitation, ocean fluxes and land surface processes. Other components such as snow and ice, underground water storage and river runoff, volcanic eruptions and aerosols, as well as products of pollution, are important but will be incorporated in the five components discussed here. These five components place the discussion of the hydrological cycle within the conceptual framework of climate research, outside the classical surface hydrology framework (precipitation, evaporation, runoff, stream discharge, soil moisture, groundwater discharge and movement).

## Clouds and radiation

Clouds, together with water vapour, influence the thermal structure of the atmosphere and the partitioning of energy between surface and atmosphere. Although globally there is a near balance in the net radiation at the top of the atmosphere when averaged over seasons, the regional distribution shows nonuniformities on all scales. These nonuniformities represent sources and sinks of energy that modulate the atmosphere and ocean circulations. Clouds play multiple roles as scatterers and absorbers of radiation. They reflect incoming solar radiation, reducing the direct solar energy input to the environment, but at the same time trap part of the Earth's emitted energy, reducing the net outflow of heat energy from the environment to space. The net effect depends on a range of cloud properties including their microphysical characteristics, their vertical and horizontal extent, and the dynamics of their interaction with the atmosphere. There is general agreement that the current, annual, global mean effect of clouds is to cool the climate system[7], but the exact magnitude and sign of cloud feedback in climate simulations is controversial because of uncertainties in predicting what types of cloud will increase as a consequence of increased $CO_2$.

**Cloud structure and dynamics.** Foremost among the microphysical properties are the size distribution, shape and phase of the cloud particles. These variables determine how the incident and emitted radiant energy in the clouds are redistributed. Numerical simulation studies[8] have shown that cloud microstructure and

TABLE 1 Sensitivity of downwelling longwave flux

| Parameter | Current uncertainties | Effect on downwelling longwave flux ($W\ m^{-2}$) Subarctic summer | Tropics |
|---|---|---|---|
| Specific humidity | 20% | 11 | 15 |
| Atmospheric temperature | 2 K | 5 | 8 |
| Cloud base height* | 100 mbar | 11 | 7 |
| Fractional cloud cover* | 0.1 | 6 | 3 |
| Doubling $CO_2$ with 0.50 cloud cover† | | 0.8 | 0.2 |
| Doubling $CO_2$ with no cloud cover† | | 1.2 | 0.3 |

Sensitivity of the downwelling longwave flux at the surface to uncertainties in atmospheric and cloud parameters and the contrast with the effects of doubled $CO_2$. Uncertainties are ± the figure given. Effects on flux are calculated by adding uncertainties to best estimate.

* Cloud base at 400 mbar.

† No feedbacks.

nucleation are important determinants in formulating cloud radiative processes. Cloud droplets (and aerosol particles), with radii from 0.001 to 10 μm, affect the radiation budget through scattering and absorption of solar and infrared radiation. Their influence is difficult to assess because concentrations vary by orders of magnitude in space and time and because observations of their size distributions, shape and chemical composition are poor. When changes in the state of the cloud water content were introduced into the model, used in ref. 8, the resulting simulations, associated with a doubling of the $CO_2$ concentration, showed a reduction of global warming from 5.2 °C to 1.9 °C. This result shows the importance of cloud microstructure and the need for a more quantitative determination of their effects.

Cloud height and albedo are also important factors. An increase in the effective cloud top height (with cloud cover and cloud albedo fixed) leads to an increase in surface temperature at all latitudes[9]. Thus, if doubling $CO_2$ leads to higher clouds, then clouds will produce a positive feedback (surface warming)[10,11]. If, however, the resulting clouds have larger water content, their albedo will be higher and they will reflect more solar radiation, thereby providing a negative feedback leading to cooling of the surface[12–14]. Special attention must be given to dimethyl sulphide emissions from the oceans, $SO_2$ emissions from fossil-fuel burning and smoke emissions from biomass burning. In the troposphere, $SO_2$ is converted to sulphate particles which, in addition to smoke aerosols, directly reflect solar radiation and act as cloud condensation nuclei[15], thereby increasing cloud albedo. The resulting cooling may nearly balance the current anthropogenic greenhouse warming[16–18].

Cloud dynamical processes play an important part in determining the vertical distribution of water vapour and the release of latent heat. Observational studies[19] of the interactions between ocean surface temperature and large-scale transport of moisture during the 1987 El Niño showed that ocean surface warming also enhanced deep convective activity and the formation of cirrus clouds with high albedo, reflecting more of the incident shortwave solar radiation. This shortwave forcing acted like a thermostat, shielding the ocean from solar radiation and regulating the sea surface temperature, capping it at a maximum value of 30 to 32 °C. Subsequent studies, however, have stressed the roles of large-scale dynamical processes[20] and evaporation[21] in regulating sea surface temperature in the Pacific.

**The cloudiness factor.** Climate models use a general parameter often called 'cloudiness' to account for the effects of clouds. When we observe the Earth from space we see certain horizontal inhomogeneities (clouds and aerosols) which we call cloudiness. This factor is quantitatively defined by measuring cloud horizontal extent or opacity relative to a specified background. The multilayer clouds shown in Fig. 2*a*[22] are derived from infrared observations in the 15-μm $CO_2$ band, and the background is defined by microwave observations in the $O_2$ cluster at 50 GHz. The resulting infrared cloud distribution is thus a measure of the cloud infrared forcing. The infrared cloud distribution is, on average, less opaque by a factor of 3/4 than the cloud distribution in the visible[23], yet shows the same basic features as the cloudiness obtained from the visible part of the spectrum.

Satellite observations of clouds provide an indispensable overview of cloud systems, letting us directly observe the effects of clouds on the Earth's radiation balance at the top of the atmosphere. The International Satellite Cloud Climatology Project (ISCCP) aims to provide detailed global cloud properties and statistics from geostationary and polar orbiting satellites. Present satellite observations cannot, however, provide information such as the height of the cloud base and the vertical structure of clouds. Current satellite observations are sensitive to the properties of clouds as seen from above whereas the longwave radiation flux reaching the surface is determined by the water vapour and cloud optical depth as seen from below.

## Atmospheric humidity

Interactions between water vapour, clouds and radiation constitute one of the most controversial feedback processes in the atmosphere. Although a very small fraction of water resides in the atmosphere, the rapid recycling of atmospheric water vapour makes it exert disproportionate control over the energetics of the climate system. Small changes in the amount of water vapour on all scales produce significant changes in cloudiness and hence radiation. Most of the atmospheric water vapour resides near the surface and its concentration varies by several orders of magnitude within the troposphere. Our knowledge of its vertical distribution and horizontal variability is uncertain, and model prediction of water vapour remains untested.

**Feedback controversies.** Raval and Ramanathan[2] used observational data to show a correlation between sea surface temperature and total water vapour, and concluded that an increase in water vapour is one of the main positive feedbacks in the atmosphere, amplifying the enhanced greenhouse effect. But Lindzen[24] argued that such a feedback may not actually develop. The warming initiated by increased $CO_2$ enhances convection and thereby changes the total water vapour and its vertical distribution. Lindzen suggested that although rapid mixing in the lower atmosphere will increase the specific humidity as surface temperature increases, greater convection will lead to decreased specific humidity in the upper troposphere, resulting in a negative feedback. Rind *et al.*[25] have observed that the water vapour density in the upper troposphere is higher in the summer season than in the winter, supporting the argument for the positive feedback of moisture. In addition, I have recently analysed data from the NOAA weather satellites (unpublished results) which show that, independent of seasons, the water content of the atmosphere increases throughout the troposphere as a function of increased sea surface temperature. This issue is still being debated, but it is certain that we need both better observations and further modelling and theoretical studies.

Despite the importance of atmospheric water vapour in weather and climate processes, most models do not make use of current data. Recent studies (E. Kalnay, personal communication) show that weather prediction models are sensitive to small changes in atmospheric moisture and that the impact of these changes on model predictions can be positive or negative depending on how the physics and dynamics of moisture processes are represented. To validate model outputs, we require consistent and more accurate measurements of humidity.

A recent approach to model validation (S. A. Clough, M. J. Iacono and J.-L. Moncet, manuscript submitted) makes use of the strong correlation between the outgoing spectral radiance to space and the spectral cooling rate. High-spectral-resolution measurements of the outgoing radiance can provide unique information about the state of the atmosphere that cannot be

deduced from broadband data. In particular, emission to space by strong water-vapour bands is a climate feedback mechanism that may be a good diagnostic of climate change. Of particular interest are the strong contributions to the radiance from the upper troposphere in the 1,400 to 1,700 $cm^{-1}$ region and from the lower troposphere in the 800 $cm^{-1}$ region. The debate about water vapour feedback might be resolved through observations with high spectral resolution (R. Goody, personal communication). Different humidity feedbacks would result in different predictions of the comparative emission of strong water lines and of medium-strength $CO_2$ lines as a function of latitude and seasons.

**Observational studies.** Observational difficulties from both space and the surface are the main hindrance in generating climatological data sets for atmospheric water vapour. Patterns of the distribution of total precipitable water vapour (Fig. 2*b*) strongly resemble the distributions of clouds (Fig. 2*a*) despite the fact that current satellite-derived moisture data is accurate only to ~10–20% over the geographical oceans and 20–30% over land[26]. Space observations of water vapour are indispensable, especially over the oceans where radiosonde observations are sparse. Even over land, problems are present. Current instruments are incapable of accurate measurement at temperatures below 230 K and at low moisture concentrations. Inconsistencies in the calibration of radiosonde data (bias changes due to changes in the sensors, and variation from country to country in processing algorithms and instrument packages) also make long-term trends difficult to establish[27]. A much more comprehensive system of monitoring atmospheric moisture is needed to derive humidity profiles throughout the entire atmosphere. Such a system would necessarily include measurements of the outgoing radiance with high spectral resolution to resolve water vapour lines[28]. In addition, improved occultation observations will be needed to measure water vapour with greater accuracy in the very highest regions of the troposphere and in the stratosphere so that radiative heating rates can be studied. Climatological data sets of atmospheric water vapour profiles derived from satellites and from upper air sondes are too poor in vertical resolution to be of much practical use. Vertically integrated water vapour (precipitable water vapour) from satellites is available but no consistent climatological data sets have yet been produced.

Poor knowledge of water vapour continues to hamper the determination of the surface radiation budget which requires, in addition to water vapour profiles, accurate knowledge of atmospheric temperature profiles, aerosols and clouds. Table 1 demonstrates the effects of current uncertainties in moisture, clouds and temperature on the computation of the downward longwave flux at the surfaces (A. Arking and M. D. Chou, unpublished results). These effects are contrasted with the effects corresponding to simple doubling of $CO_2$ (with no feedbacks) for two cases: 50% clouds and no clouds. Because of current uncertainties, the errors in calculating the downwelling longwave flux remain too large (by about one order of magnitude) to let us determine the impact of increased $CO_2$ on global warming.

## Precipitation

The average annual global precipitation is equivalent to 95–115 cm each year or about 0.3 cm per day. But precipitation is highly variable, with two-thirds of the global precipitation occurring between latitudes of 30° N and 30° S. This variability not only has a tremendous influence on vegetation, droughts and floods but also has a controlling effect on the large-scale circulation of the atmosphere and oceans. In spite of this extreme variability, long-term, time-averaged precipitation fields reveal large-scale patterns that are of great significance in maintaining the hydrological cycle and the climate system.

The atmospheric forcing caused by spatial variation in the release of latent heat of condensation is the main driver of the dynamics of the interactions between the atmosphere, oceans and land. The annual variability of tropical rainfall is strongly related to the annual variability of the sea surface temperature, reflecting the strong coupling between the ocean and the atmosphere. The three key parameters controlling this coupling are sea surface temperature, rainfall and wind stress. Even though the amplitude of the annual cycle of sea surface temperature is small, the thermal coupling is strongly amplified by the latent heat released by large- and small-scale regimes of precipitation. This influences the surface wind field, which changes the ocean upwelling and current system and, in turn, induces further changes in the sea surface temperature in an interactive feedback loop.

These interactions are frequently associated with the evolution of monsoons, tradewind systems and oceanic convergence zones, and with the El Niño/Southern Oscillation (ENSO) cycle which occurs at intervals of 2 to 7 years[29]. The ENSO warm phase in 1991–92 was preceded by one in 1986–87 and another in 1982–83, the strongest this century. In general, ENSO is the most noticeable case of climatic hydrological variability, affecting marine ecosystems along the west coast of America as well as regional and global food production. There is also considerable coherent atmospheric variability on timescales of ~40–50 days, which markedly resembles the low-frequency ENSO cycle[30]. These oscillations are particularly evident in rainfall and wind fluctuations over the low-latitude tropics, from the eastern Indian Ocean to the central equatorial Pacific[31].

Tropical rain systems affect both the upper troposphere and the lower stratosphere. Convective tropical rain provides the energy for fluxes from the troposphere to the stratosphere, known as the 'stratospheric fountains'[32]. The advection of water vapour into these regions provides the moisture needed for the formation of tropical cirrus clouds. From these tropical regions moisture spreads worldwide.

Over land, understanding the interactions between rainfall and surface processes is important on all scales, from microscale ($1 \times 1$ $km^2$) to continental. For example, there is a high degree of recycling between rainfall and evapotranspiration in regions like the Amazon, whereas in desert areas the absence of significant sources of surface water vapour may help to maintain the desert itself[33]. In the latter case, it is believed that the dry conditions are initiated by large-scale atmospheric factors and reinforced by surface processes.

**Precipitation data.** Precipitation is one of the most difficult processes to model and predict. Cloud microphysics and particle growth, as well as the regional and global patterns of temperature, humidity and winds, control the intensity, scales and timing of rainfall. Figure 3 illustrates the uncertainty in our knowledge[34]. The solid and dashed curves represent best interpretations from rain-gauge measurements. The other symbols refer to the results of numerical models. The models succeed in reproducing the extensive rainfall in the tropics but differ in determining the latitude and magnitude of the peak. Differences as large as a factor of five occur in their prediction of the global monthly rainfall at the peak point. Regionally, the variations between the leading models are even worse[35]. The simplistic parameterization of rain processes in global models is the main reason for these poor results. Efforts to improve these models are hampered by the lack of reliable data.

New analyses of infrared and microwave data from space are being undertaken to fill the gap in our knowledge of the global distribution of precipitation. Visible and infrared data are most commonly used today for estimating precipitation. Because precipitation is a product of a complex combination of thermodynamic and dynamic processes, it is possible to formulate a rainfall index from features of cloud and radiation fields derived from satellite observations and then relate the index, through regression equations, to rain-gauge observations of rainfall.

The Tropical Rainfall Measurement Mission[36] is a joint satellite mission between Japan and the USA. It will be launched (around 1997) into a low-inclination orbit from which it will

observe mostly ocean areas, and will fly a complement of four instruments: a radar, a microwave radiometer, a visible-infrared radiometer and an Earth radiation budget instrument. The results should establish the necessary link between deterministic and stochastic models of rainfall fields. We need global measurements of precipitation over both land and oceans to close the hydrological cycle by accurately determining the partition of precipitation between ocean and land areas, thereby improving the representation of the balance between runoff and evaporation[37].

## Ocean fluxes

The oceans contribute to the global hydrological cycle in two ways. First, they provide long-term memory by storing heat and releasing water vapour. Second, in the subtropics the oceans transport heat polewards at a rate equal to the heat transported by the atmosphere. Improved understanding of the interactions of the oceans with the atmosphere will, it is hoped, allow us to describe and predict the behaviour of the upper oceans accurately enough to close the global hydrological cycle. Oceans move both horizontally and vertically under the influence of winds and density differences. These density differences are related to changes in water salinity which can be generated by evaporation, precipitation, runoff and ice melting. The combination of low surface temperature and high salinity makes water more dense than the deep water below, setting up convection which affects even the deep ocean layers.

**Heat transport.** Salinity modulates the interactions of the oceans with the hydrological cycle. In the thermohaline circulation of the northern Atlantic Ocean, warm surface water flows northward, becoming more dense through increased salinity and cooling. It then sinks and flows south. The addition of fresh water reduces the density of the upper ocean layers and represses sinking, thereby creating a cap over the ocean which could eventually lead to vastly different thermohaline circulation patterns[38]. Lower surface salinity in the northern Atlantic could be brought about by increased precipitation, melting of the ice caps or changes in the flow of low-salinity water from the Arctic Ocean. This affects the transport of heat to high latitudes in the Atlantic basin and possibly the upper ocean currents over the world. Model simulations indicate that small changes in the Atlantic thermohaline circulation could have prolonged effects on the whole climate system[39].

Despite its importance, the fresh water flux at the ocean surface is poorly known. Rainfall measurements over the oceans are almost non-existent and evaporation is typically estimated from an empirical 'bulk' formula requiring ocean surface temperature, near-surface humidity and wind speed[40]. The difference between these poor estimates of evaporation and precipitation is used to derive even poorer estimates of the distribution of fresh water at the ocean surface, except across international shipping routes in the North Atlantic[41].

Present estimates of heat transport by the oceans are deficient in other ways, resulting in a discrepancy known as the 'mystery' of the missing 1.6 pW (1 pW = $10^{15}$ W) in the heat balance of the climate system[42]. The poleward transport of heat at 24° N by the Pacific and Atlantic oceans has been estimated[43] to be 2.0 pW (the Indian Ocean does not extend past latitude 24° N, so it does not have to be considered here). This is comparable to the value of 1.7 pW estimated for the heat transport by the atmosphere[44]. The oceans and the atmosphere together thus transport 3.7 pW northwards, far less than the 5.3 pW required to balance the Earth radiation budget according to satellite data[45].

**Ocean observations and modelling.** Modelling the effects of atmospheric forcing on the upper ocean layers is very different from modelling interactions between the atmosphere and land surface, because of differences in the rates of vertical and horizontal transfer of heat and fresh water in the two media. In the extratropics, the fluxes of water vapour and energy from the ocean surface to the atmosphere are strongly affected by ocean eddies on a scale of about $100 \times 100$ km$^2$; unless climate models can deal with these scales they cannot closely reproduce the larger motions. To improve the models further we need to increase their resolution and refine the parameterizations of heat transport by ocean currents, deep convection and other aspects of the annual cycle. Two current experiments addressing these issues are the Tropical Ocean Global Atmosphere (TOGA) experiment and the World Ocean Circulation Experiment (WOCE). Together, these will provide a more comprehensive understanding of the hydrological cycle over the oceans.

## Land surface hydrological processes

Studies of land surface interactions must address surface inhomogeneities on all scales. Figure 2*c* illustrates an aspect of this inhomogeneity: it shows the difference between day and night temperatures of ocean and land surfaces. Because water has a high heat capacity, the oceans show little change in temperature between day and night. Dry land surfaces such as deserts show extreme differences in temperature, whereas wet, forested or vegetated areas show intermediate levels of change in temperature between day and night.

Evaporation and precipitation over land are major components of the global hydrological cycle. Energy is needed to convert soil water to vapour. Most of this energy comes from radiation absorbed by the surface, which depends on surface albedo and other factors. Surface albedo itself is determined by vegetation and bare soil conditions. Changes in vegetation and soil moisture alter the partition between evaporation and runoff which, in turn, changes surface conditions. Land surfaces affect

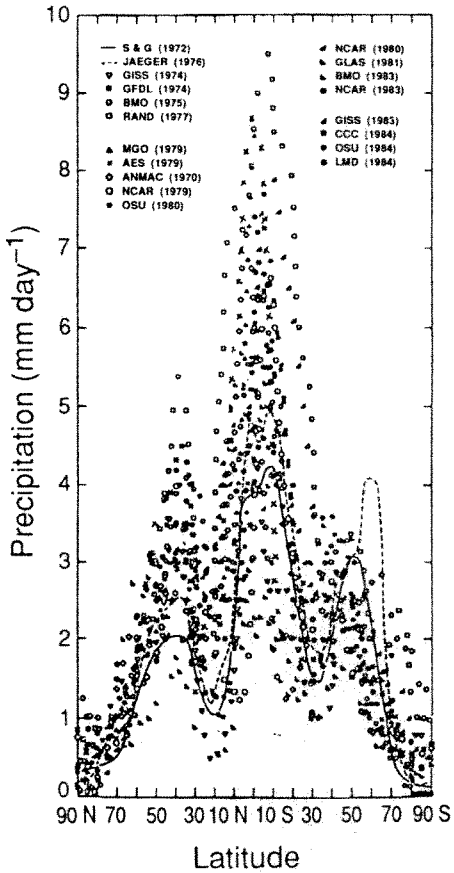


FIG. 3 Mean zonally averaged precipitation for January (see ref. 34 for details of models). The uncertainty in the estimates of global precipitation is typified by the scattering of the data between rain-gauge measurements and among simulations by different models. The solid and dashed curves were put together by different authors from surface-based rain-gauge data. The other symbols refer to results from some of the best models.

the hydrological cycle in various additional ways. For example, in the extratropics, which experience large seasonal fluctuations, the soil stores some of the precipitation it receives in winter and returns it to the atmosphere in summer. Snow and land ice must be included in land surface parameterization[46]. This is particularly important in high latitudes where they have a considerable effect on surface heating. Because of the multiple interactions between climate and surface conditions it is difficult to foresee the effect of human-induced changes (such as deforestation or changes in land use) on either local or global-scale climate.

A few quantitative studies have been made of the effects of land surface processes, using atmospheric GCMs[47]; they indicate that these processes are essential in maintaining climate and controlling its temporal variation.

**The physical aspects.** Given a knowledge of soil properties and moisture content, the most important surface parameters determining evaporation are albedo and roughness length. The first soil model was the reservoir representation by Manabe[48], known as the 'bucket' model. This model is limited by a moisture capacity of 15 cm. Precipitation in excess of this value becomes runoff. In Manabe's model, evaporation proceeds first at the potential rate (the atmospheric vapour transport capacity) until a critical value of soil moisture is reached, then continues at a linear rate proportional to the soil moisture. In reality, the relationship between rainfall, runoff and evaporation is highly nonlinear and requires more complex representation schemes[49]. Hanson *et al.*[50] introduced geographical variations in surface albedo, roughness length and root depth. Dickinson[51] and Sellers *et al.*[52] developed more elaborate schemes. Soil models must now be combined with their counterparts for vegetated surfaces before testing in a GCM.

**The biological aspects.** Vegetated surfaces complicate the problem of representation of the land surface[53]. For plant transpiration, the shape and physiology of vegetation are important. Morphology determines the absorption and reflection of radiation and the physiology controls the latent heat flux.

Although the need for GCMs to include vegetated surfaces is well accepted, attempts have so far been limited. Dickinson[51] and Dickinson *et al.*[54] proposed the biosphere–atmosphere transfer schemes, and Sellers *et al.*[52] proposed the simple biosphere model. These models are based on the 'big leaf' concept[55], in which the vegetation is represented by a layer of negligible heat capacity. In model grid cells with incomplete vegetation cover, the model assigns a proportion of the cells to bare soil and others to vegetation; thus the determination of surface energy flux of sensible and latent heat includes contributions from both bare soil and vegetation canopy.

Recent studies[56–58] have tested climate model responses to deforestation in Amazonia. Vegetation and soil characteristics were changed to represent those typical of deforested regions in Amazonia. Evaporation over the deforested region decreased as a result of increased albedo. The first results[56] showed little effect on the overall precipitation but later work[57,58] indicated a larger reduction, suggesting sensitivity to model formulation.

**Land surface observations and modelling.** Realistic modelling of land surface processes is essential for successful simulation of climate[59], but there are many obstacles. To improve land surface hydrological models for GCMs we must now learn how to combine the effects of processes from local to GCM grid scales in a rigorous manner. Analyses of data from coordinated field experiments will assist our progress in this area[60].

## Global data and space observations

Satellites will have an important, but by no means exclusive, role in the observation of the hydrological cycle. Global observations must be a combination of traditional operational weather data and future Earth-observing satellites. The required data fall into five specific categories: basic meteorological parameters such as temperature and moisture; tropospheric wind vectors; precipitation; radiation and clouds; and land surface data. In each of these areas, new instrument capability is needed: high-spectral-resolution infrared spectrometers for high-vertical-resolution sounding of atmospheric temperature and humidity; Doppler lidar measurements of tropospheric winds; global measurement of precipitation from space, using rain radar; determination of the complete hemispheric distribution of the Earth's radiance and its angular distribution by means of a radiation budget radiometer, as well as cloud base height and vertical structure from radar measurements; and high spatial resolution observations of surface vegetation and cloud structure by means of multispectral imaging.

## Emerging new community

Determining the manifestation of future climate change begins with assessing our understanding of the components of climate. Progress is being made, but much remains to be done. Even if great accomplishments are made in each of the separate areas discussed here, they must still be integrated to solve the problem of climate change.

In the short span of about 10 years, the hydrological cycle has emerged as the centrepiece of the study of climate, but basic changes are still required in this field. Hydrological science must adjust itself to become a discipline not unlike atmospheric science or oceanography. Rather than fragmented studies in engineering, geography, meteorology and agricultural science, we need an integrated program of fundamental research and education in hydrological science. As Frank Press, president of the US National Academy of Sciences, has stated: "the scientific and educational base in hydrology is incompatible with the scope and complexity of many current and emerging problems"[61]. The shape of the emerging discipline is still evolving[62]; there are difficult mental adjustments to be made, and the transition has only just begun. □

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# Continuum of overlapping clones spanning the entire human chromosome 21q

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**A continuous array of overlapping clones covering the entire human chromosome 21q was constructed from human yeast artificial chromosome libraries using sequence-tagged sites as landmarks specifically detected by polymerase chain reaction. The yeast artificial chromosome contiguous unit starts with pericentromeric and ends with subtelomeric loci of 21q. The resulting order of sequence-tagged sites is consistent with other physical and genetic mapping data. This set of overlapping clones will promote our knowledge of the structure of this chromosome and the function of its genes.**

HUMAN genome mapping consists of ordering genomic DNA fragments on their chromosomes using several methods, such as fluorescence *in situ* hybridization (FISH), somatic cell hybrid analysis or random clone fingerprinting[1–10]. When the fragments correspond to polymorphic sites they can be ordered by genetic linkage analysis[11]. Distances between polymorphic loci are estimated by meiotic recombination frequencies. Such a genetic map allows the localization of any polymorphic trait gene.

Human chromosome 21 (HC21) represents a model for physical mapping of the human genome and is the smallest and one of the best-studied human chromosomes. Several genetic diseases are associated with this chromosome[12], including Down's syndrome (the most frequently occurring mental retardation in humans), some forms of Alzheimer's disease and other neurological diseases, such as progressive myoclonus epilepsy and amyotrophic lateral sclerosis. A map of contiguous units (contigs) covering this chromosome will speed the identification of the cause of these diseases. Indeed, it provides an immediate access to the genomic segment, including any pathological locus, as soon as it has been localized by genetic linkage or cytogenetic analysis.

The process of developing such a long-range contig map involves the identification and localization of landmarks in cloned genomic fragments. When there are enough landmarks for the size of the cloned fragments, contigs are formed, and the landmarks are simultaneously ordered[13]. Yeast artificial chromosome (YAC) cloning provides the means to isolate large, but manageable, DNA fragments of 100 to 2,000 kilobases (kb);

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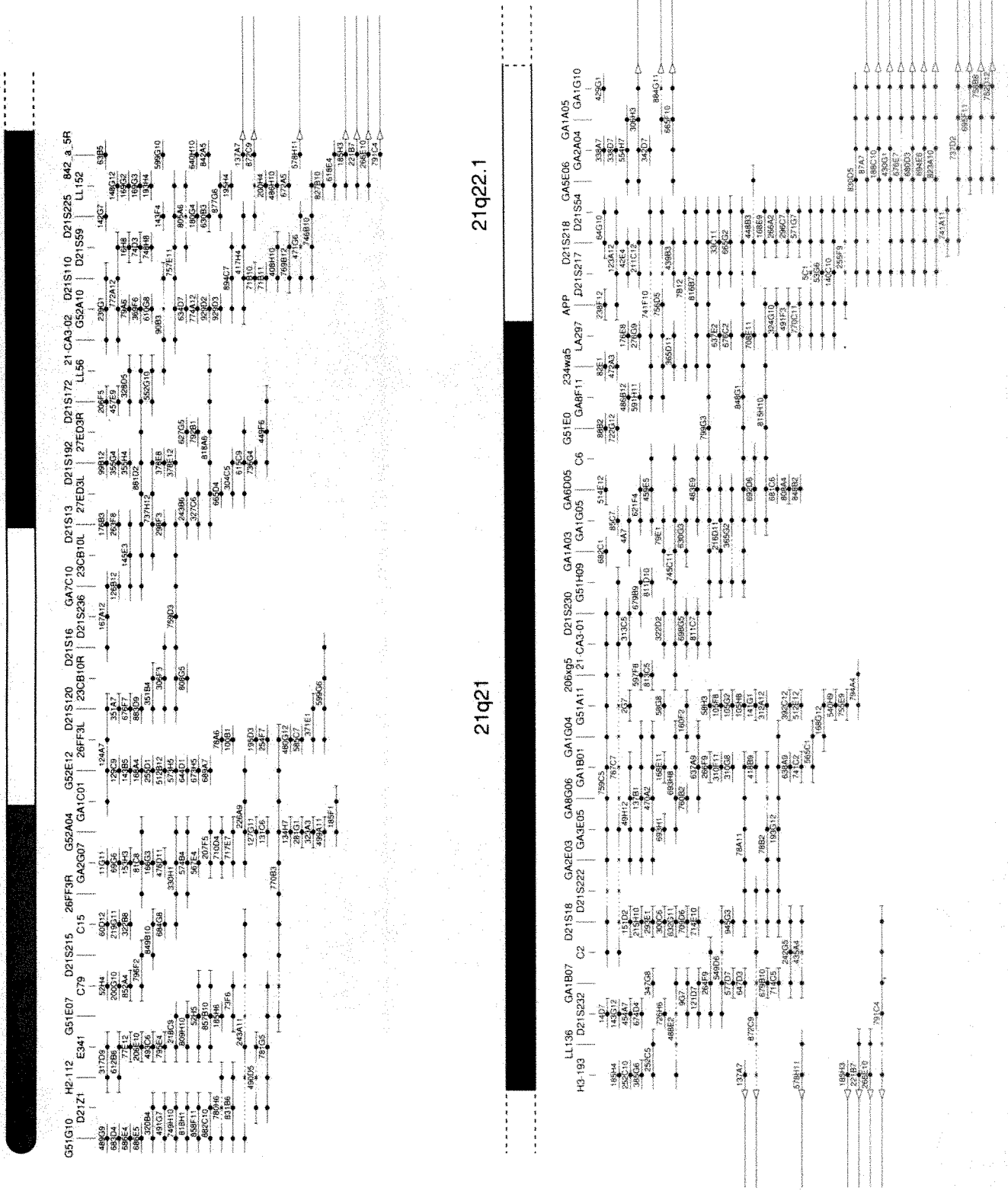



FIG. 1 Above and pages 382/3. STS content map of the contiguous array of YAC clones. Clones are presented as lines. Their size only reflects the number of included STSs. The physical distances between adjacent STSs are diverse. As the degree of chimaerism of each clone is unknown, only HC21-specific portions are represented. Filled circles indicate positive STS. Broken lines mean that a clone was not found to be positive with a given STS when only screening pools. A cross means that a clone, when checked individually, was found to be negative with an STS. Bars at the end of the clone indicate that a clone was found to be negative with an adjacent STS. For comparison, a drawing showing G-banded chromosome 21q is placed above the contig presentation.

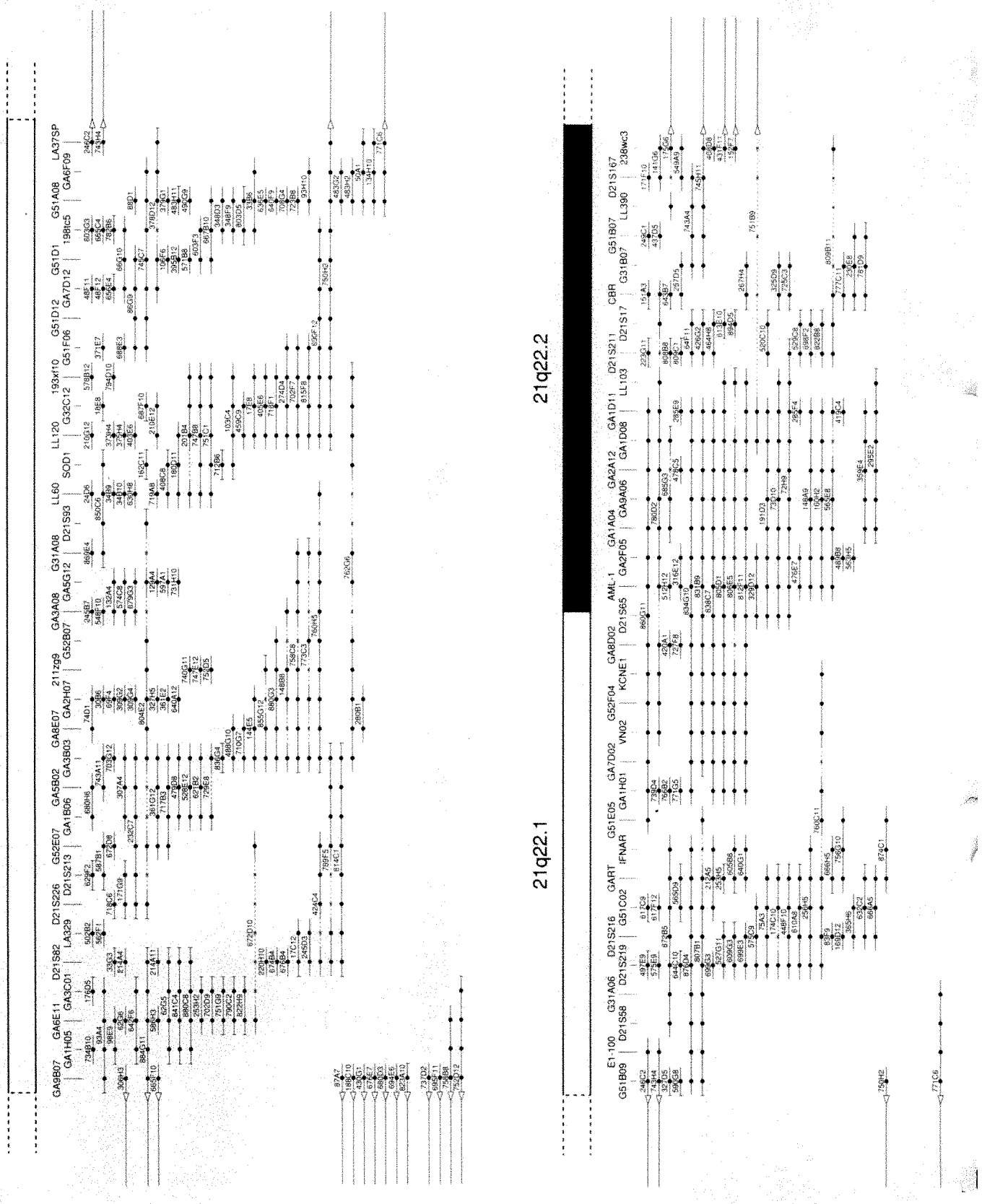



FIG. 1—continued.

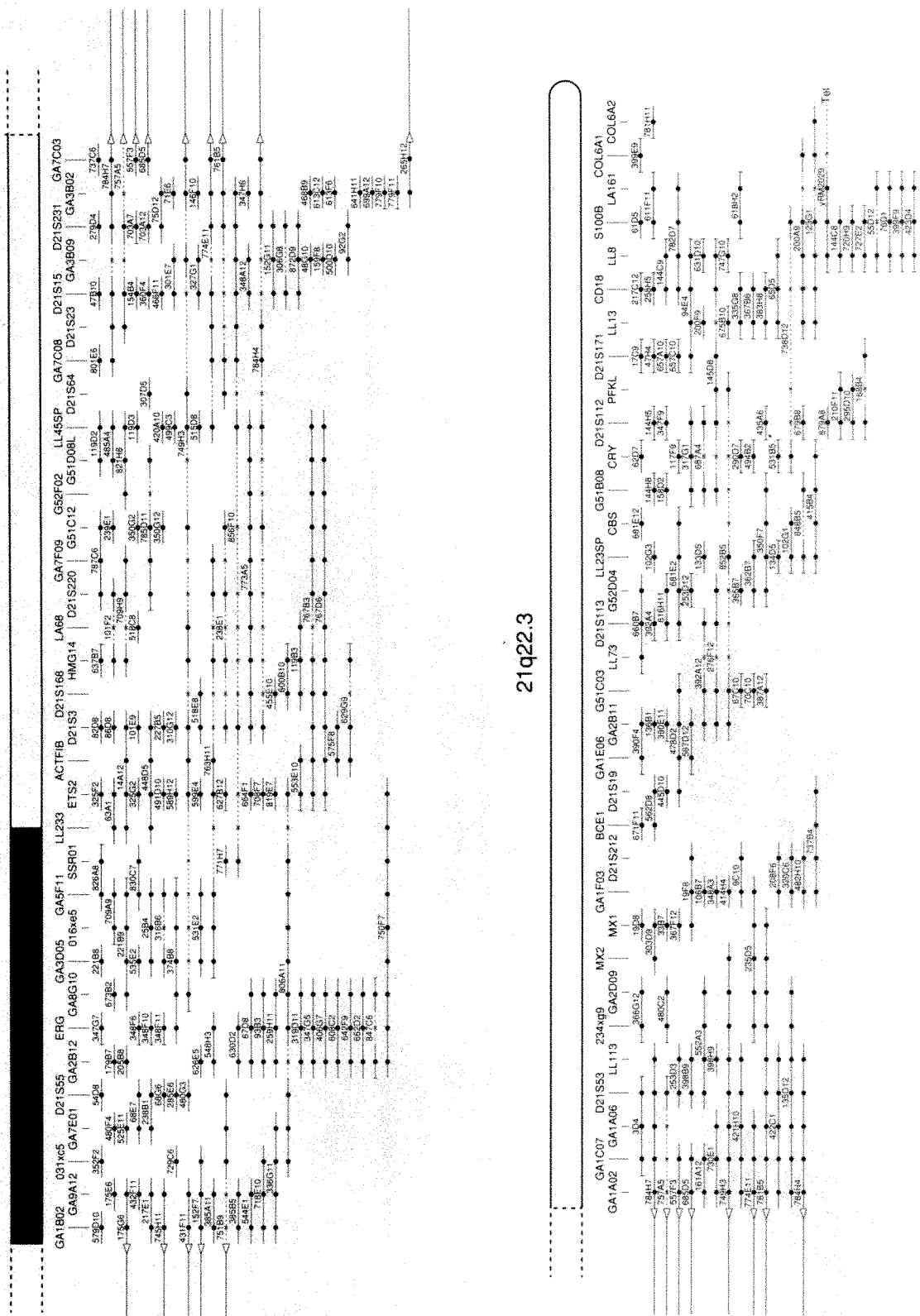



FIG. 1—continued.

these are much larger than those isolated using bacterial cloning systems[14-18]. This fills the gap between genetically and physically measurable distances. Moreover, the moderate size of YAC libraries make them well-adapted to screening by polymerase chain reaction (PCR). This allows the use, as landmarks, of sequence-tagged sites (STS)[19], which are short stretches of DNA sequence that can specifically be detected by PCR. Implicit in the definition is that the STS is operationally unique in the genome. A major advantage of this strategy is that it provides an ordered set of markers that can be stored as sequence information in a database. Other potentially informative physical landmarks can be readily converted into STS by DNA sequencing. They establish the common language for the comparison with other physical mapping data. Moreover, ordered collections of clones can be reconstituted from new libraries, when using ordered STS, providing an easy update as cloning technology improves[19]. We report here the first STS map for the entire human chromosome 21q (HC21q) that is now covered by a single array of overlapping YACs with a mean size of about 600 kb.

## A set of 21q-specific sequence-tagged sites

Sequences of several HC21 genes are available from databases. These sequences can easily be converted into STS. Recently a number of STS, mainly polymorphic, were generated for this chromosome to aid genetic linkage studies. Other STS include markers from *Not*I-linking clones[20] and from the vicinity of naturally occurring chromosome translocations. Because there is a tendency of some of these markers to be nonuniformly distributed throughout the length of the chromosome, we also screened with other anonymous STS from different origins. One of these sources is the recent collection[21] of STS derived from previously mapped cloned fragments. We also converted some cloned PCR products from flow-sorted HC21 preparations into STS. This gave us 88 new anonymous markers, the largest single source of STS. All of them have been submitted to the Genome Data Base, the full list being available on request. In total, we have 21 STS derived from *Not*I-linking clones, 50 polymorphic STS and 21 STS derived from known genes, whereas the remainder are mainly anonymous markers. Some STS, assigned to HC21, could not be efficiently used for the screening because they produced nonspecific amplication from the yeast DNA or primer dimerization. All STS, whatever their source, were tested on a somatic cell hybrid containing only HC21 (ref. 22) before use and, in most cases, on another hybrid, containing only HC21q (refs 21-23).

## Screening YAC libraries and contig assembly

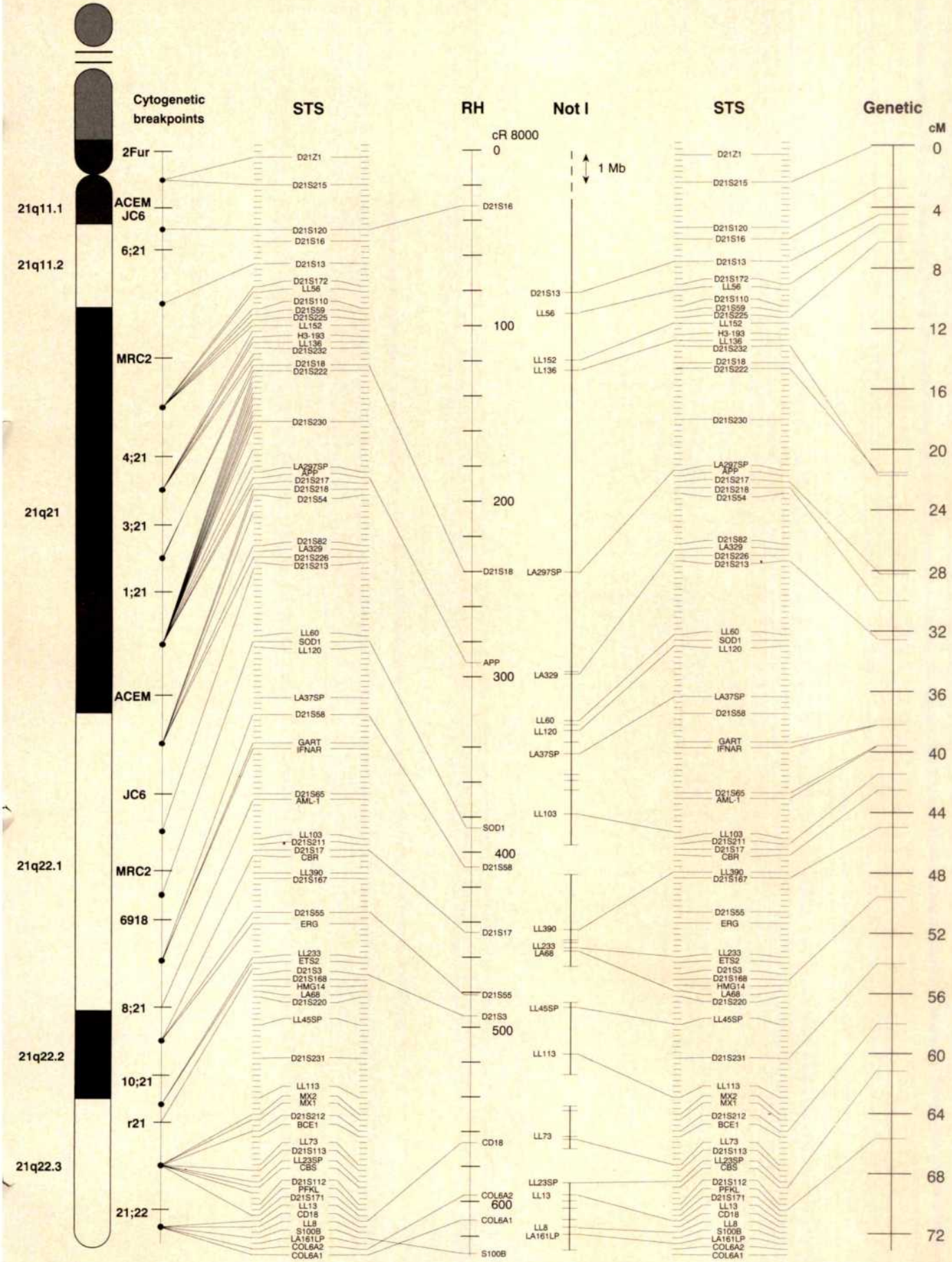
Three different YAC libraries were screened entirely by PCR for the presence of the HC21q-specific STS. The first one, containing about 70,000 YAC clones of average size 470 kb, corresponds to 9.4 human genome equivalents. It was made from the DNA of the same lymphoblastoid cell line and used to construct a subset of this library of about 50,000 clones already described[17]. This library, stored as an array of individual clones in 736 96-well plates, was screened by PCR in a two-step pooling procedure. We first tested 92 primary pools (8 plates each) representing all clones in the library. The candidate clone in a given positive primary pool was identified by testing 28 smaller pools. These smaller pools contained DNA from clones of individual plates (8 pools), rows (8 pools) and columns (12 pools) of the corresponding 8-plate set. On average, 355 tests were necessary to identify the clones positive for a given STS from this 9.4 genome-equivalent library. To exclude false positives, we usually checked candidate positive clones individually. Another YAC library, chromosome 21-specific, consisting of 180 clones was derived from 14,000 YACs of 1 megabase (Mb) mean size, as previously described[18]. This sublibrary corresponds to about four genome equivalents. Its clones were screened individually. Both total human genome libraries (P.O., manuscript in preparation) were constructed using *Eco*RI partially digested genomic DNA, sized through pulse-field agarose gel electrophoresis[17] and inserted into a pYAC4 vector[14]. The last library[24], consisting of human telomere-containing YACs, was only screened with subtelomeric STS. Analysis of PCR reactions was done by agarose gel electrophoresis and images were directly read by a CCD (charge-couple device) camera, then processed by appropriate software and transferred on-line to a specially designed SYBASE database. A special algorithm (available on request from the authors) was developed to produce and order contigs and landmarks. For a given STS order, an energy function was calculated, taking into account all inconsistencies in the clone overlaps. The best order was proposed by minimizing this energy function by simulated annealing[25]. This kind of ordering may occasionally lead to wrong solutions mainly due to the presence of false positives and negatives. But we assume that this problem can be overcome by using a sufficiently redundant library.

## Continuum of YACs on 21q

In total, for 198 STS used to screen a 13.4 human genome-equivalent library we isolated 810 positive clones. The mean number of positive YACs per STS was 10.2. Although comparable to the library redundancy, this slightly lower number probably reflects overall efficiency of our screening procedure and also the presence of deleted clones. When corrected for the clones that are apparently deleted (at most 12%) this number increases to 11.2.

The array of overlapping clones is shown in Fig. 1. It contains the positive clones for all STS used for the screening. The interval between two STS is covered by an average of five clones, indicating the robustness of the contig. There are two main sources of error in contig assembly. We tried to exclude trivial false positives, by checking candidates individually. False merging could also be due to so-called chimaeric clones that contain more than one genomic region. Owing to the small size of HC21 (1.5% of the human genome), the frequency of such HC21 to HC21 chimaeric clones is only 0.7%, calculated on the basis of 40% chimaeric clones in the overall library (P.O., manuscript in preparation). But given the large redundancy of the library, problems due to potential chimaeras can be resolved (indeed we observed only one clear case of such a problem). Another source of false overlap can be inclusion of data from PCR landmarks that are not STS in a strict sense (not unique). This problem can be solved partially by assignment of STS to human chromosomes using a somatic cell hybrid mapping panel. We also considered a particular PCR landmark as a moderate repeat when it gave too many positive primary pools during the first step of screening. STS that gave more than 15 positives when tested on primary pools from the 9.4 genome-equivalent library were usually excluded from subsequent screening. Nevertheless, the six most proximal STS of our contig are also present on chromosomes other than HC21. PCR landmark D21Z1, a pericentromeric alpha satellite repeat[26], is present on chromosomes 21 and 13. We have shown that the STS E341 (ref. 27), derived from the sequence of minicircle DNA is present on chromosomes 21, 15 and 22. Polymorphic marker D21S215 also has a nonpolymorphic counterpart on chromosome 18. The rest of the markers in this region (G51G10, G51E07 and C79) are specific to other acrocentric chromosomes (13, 14, 15, 21 and 22). It is possible, therefore, that single STS-positive clones and even some of the STS-linking clones in this region do not

FIG. 2 Comparison of the STS map to the breakpoint panel map, irradiation hybrid map, *Not*I fragment PFGE map and genetic linkage map. STS are represented as bars, each corresponding to an individual site. Only some that are essential for comparison have been named. Scales are given in centirays (cR) for the radiation hybrid map, megabases (Mb) for PFGE map or centimorgans (cM) for genetic data. ►


Cytogenetic breakpoints
STS
RH
Not I
STS
Genetic
cM
cR 8000
1 Mb
2Fur
ACEM
JC6
6;21
MRC2
4;21
3;21
1;21
ACEM
JC6
MRC2
6918
8;21
10;21
r21
21;22
21q11.1
21q11.2
21q21
21q22.1
21q22.2
21q22.3
D21Z1
D21S215
D21S120
D21S16
D21S13
D21S172
LL56
D21S110
D21S59
D21S225
LL152
H3-193
LL136
D21S232
D21S18
D21S222
D21S230
LA297SP
APP
D21S217
D21S218
D21S54
D21S82
LA329
D21S226
D21S213
LL60
SOD1
LL120
LA37SP
D21S58
GART
IFNAR
D21S65
AML-1
LL103
D21S211
D21S17
CBR
LL390
D21S167
D21S55
ERG
LL233
ETS2
D21S3
D21S168
HMG14
LA68
D21S220
LL45SP
D21S231
LL113
MX2
MX1
D21S212
BCE1
LL73
D21S113
LL23SP
CBS
D21S112
PFKL
D21S171
LL13
CD18
LL8
S100B
LA161LP
COL6A2
COL6A1
0
100
200
300
400
500
600
D21S16
D21S18
APP
SOD1
D21S58
D21S17
D21S55
D21S3
CD18
COL6A2
COL6A1
S100B
D21S13
LL56
LL152
LL136
LA297SP
LA329
LL60
LL120
LA37SP
LL103
LL390
LL233
LA68
LL45SP
LL113
LL73
LL23SP
LL13
LL8
LA161LP
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72

originate from chromosome 21. But at least clones 243A11, 490D5, 781G5, 770B3, 796F2 and 849B10 establish the overlap of HC21-specific clones from the alpha satellite PCR marker D21Z1, localized at the centromere, to the HC21q-specific marker C15. From this point, the contig extends uninterruptedly and ends with the clone yRM2029, that contains active human telomere and three subtelomeric STS markers.

In some regions of our contig, the linkage is mediated only by a few or by unstable clones. We used repeat-containing restriction fragment fingerprint[9] (C.B.-C. *et al.*, manuscript in preparation) and Alu PCR product patterns[18] (I.C., unpublished results) of YACs to confirm these regional clone overlaps. Using these methods we were able to document the linkage in the regions between LL45SP and D21S64, LL56 and 21-CA3-02, LL136 and D21S232, LL103 and D21S211 (data not shown). The case of apparently uncertain linkage in the vicinity of LL73 is due to the low reproducibility of amplification obtained with the primers corresponding to this STS, the weakness disappearing when it is omitted.

It is difficult to estimate the effective genomic length of a contig produced by STS content analysis. Some of the STS used were derived from sequences around *Not*I sites. The lengths of the clones covering these intervals are compatible with the size of corresponding *Not*I fragments (data not shown). The sum of fragments, produced by *Not*I from the 21q suggests a size between 40 and 50 Mb[20,23]. A very rough statistical estimate for the size of our contig, derived from the total number of positive clones, their average insert size and corrected for the estimated 40% chimaeras gives a figure of the same order (42 Mb). Moreover, although it is difficult at present to define a minimal set of overlapping YACs, we estimated their number to be 55 with a mean size of 1 Mb.

## Order of STS landmarks

Construction of the YAC contig by STS content analysis simultaneously produces the relative order of these landmarks across the chromosome. The resolution of this map is dependent not only on the number of STS tested, but also on the number of YACs containing informative combinations of STS. The most useful are those containing only pairs of adjacent landmarks. We found that, although the contig assembly was more efficient with larger YACs, the STS map was more accurate with smaller clones. The landmark order resolution is refined when the clone coverage increases. Obviously in certain regions, corresponding to an exceptionally high density of STS (for example in the vicinity of AML1 (acute myelogenous leukaemia)) no local order can be unequivocally deduced, given the lack of informative clones. Another factor influencing the order of STS is the presence of clones, not detected with a given STS, but detected with its neighbour. Such false negatives can create local errors when deducing order. They can be eliminated by checking clones for the presence of neighbouring STS. We found that in some regions these tests do change regional STS order. The present map reflects the number of such tests done. Another problem is the presence of rearranged, unstable clones creating errors which are difficult to eliminate when they occur at the same site. One such hot-spot for deletions is located between D21S3 and D21S15, another one being evident in the subtelomeric region. This latter could explain, for example, the more distal position proposed for the phosphofructokinase liver-type marker, relative to its immediate neighbour D21S112.

A few markers mapped at several places on our contig. For example, D21S11 has two possible locations: one close to D21S110, the other close to D21S232. LL54 is localized close to D21S18 and in the vicinity of $\beta$-amyeloid precursor protein. The simplest explanation is the existence of homologous regions in this part of chromosome 21. This also illustrates the potential difficulties that certainly will be encountered in the mapping of other genomic regions using the STS approach.

## Comparison with other maps

The order of markers for chromosome 21 was derived by several other methods, including genetic linkage analysis[28] (M. G. McInnis *et al.*, manuscript in preparation), pulse-field electrophoresis analysis of fragments produced by rare-cutter endonucleases[19], the analysis of panels of somatic cell hybrids, containing naturally occurring deletions[23] or radiation-induced deletions[3]. Some of the markers used in these studies available as STS were mapped in our contig. The comparison of their position to known genetic and physical maps is given in Fig. 2. Most markers show the same relative order when all of these maps are integrated. The few discrepancies include the relative order of most telomeric markers on the radiation hybrid map. This can be explained both by the differential mode of retention of subtelomeric regions in radiation hybrids and by the abnormal nature of YACs derived from the telomere. Certainly we have a few clones containing genes COL6A1 and COL6A2, whose absence in human telomeric YAC yRM2029 argues for their more proximal position, concordant with radiation hybrid mapping. Our map fits with the orientation of LL390, LL233 and LA68 as it is drawn on the *Not*I map. Another possible inverted orientation of these markers has recently been suggested (H.I., M.O., unpublished results), but would be inconsistent with the integrated STS and genetic map in this region. Another *Not*I STS, LL102, is also located 100 kb from AML1 in the region of the above mentioned cluster of high-density STS.

## Discussion

This work provides an essential tool for improving our knowledge of HC21, especially to find new genes and, more generally, to derive sequence data. But the choice of clones from which such information could be generated remains problematic owing to the frequency of chimaeric and rearranged inserts inherent in the cloning system. Size information, already obtained in 70 YACs did not yield a definite conclusion concerning these cloning artefacts. Therefore, future work on HC21 will certainly require checking by several methods, such as high-resolution restriction mapping. Moreover, new clones obtained from other cloning systems can rapidly be aligned to the present contig. Indeed, other libraries can be screened with these STS and other probes derived from this collection of YACs.

In total, 198 landmarks used were resolved in 191 discrete loci with an average spacing of roughly 220 kb. Only six loci contain more than one STS, five with two STS and one with three. This resolution probably exceeds the accuracy of most mapping approaches. Indeed, from such arrays of YAC clones, new polymorphic markers can be generated directly from YACs to create a high-resolution genetic map, allowing mapping of genes involved in multifactorial traits.

Despite its small size, chromosome 21 might not be much different in structure from other human autosomes. Construction of a YAC contig for its entire length indicates clearly that STS content mapping could also be applied to other chromosomes. It also demonstrates that this strategy can be used directly, without regional assignment of landmarks by other means. It is tempting, therefore, to suggest that such an approach could be applied to the entire human genome using completely random landmarks. □

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# Crystal structure of the *met* repressor–operator complex at 2.8 Å resolution reveals DNA recognition by β-strands

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**The crystal structure of the *met* repressor–operator complex shows two dimeric repressor molecules bound to adjacent sites 8 base pairs apart on an 18-base-pair DNA fragment. Sequence specificity is achieved by insertion of double-stranded antiparallel protein β-ribbons into the major groove of B-form DNA, with direct hydrogen-bonding between amino-acid side chains and the base pairs. The repressor also recognizes sequence-dependent distortion or flexibility of the operator phosphate backbone, conferring specificity even for inaccessible base pairs.**

GENE regulation both in prokaryotes and in eukaryotes is achieved largely by proteins that bind to specific sequences in the DNA. The source of this specificity is recognition by the protein of the pattern of functional groups on the edges of the base pairs in the DNA major groove, mediated by hydrogen-bonding, together with a contribution from the sequence-dependent conformational preferences of the DNA backbone[1,2]. Structural studies of a number of prokaryotic repressors and activators[3–13], and eukaryotic homeodomain complexes[14,15], have demonstrated that insertion of one of the α-helices of a conserved helix-turn-helix motif into the major groove is a frequent method of interaction. The structures of two types of zinc-finger–DNA complexes[16,17] also show α-helices lodged in the major groove. We report here the crystal structure of the *Escherichia coli met* repressor–operator complex, where β-strands rather than α-helices interact with the DNA bases to mediate sequence recognition.

Expression of many of the structural genes in the methionine biosynthetic pathway of *E. coli* (reviewed in ref. 18) is controlled largely by the *met* repressor protein, the product of the *metJ* gene. One of the products of the pathway, *S*-adenosylmethionine (SAM), acts as a corepressor *in vitro* and, presumably, *in vivo*. The repressor is a dimer ($M_r$ 23,992) of identical 104-amino-acid subunits, which binds two molecules of SAM non-cooperatively; its three-dimensional structure has already been reported in the presence and absence of corepressor[19]. The repressor binds cooperatively to a number of operators, all of which share sequence homology to an underlying 8-base-pair (bp) repeating unit (AGACGTCT), referred to as the '*met* box', in two to five tandem copies[20–22]. We proposed[21] that arrays of dimeric *met* repressor molecules bind to these extended operator regions, with a stoichiometry of one repressor per *met* box, to form a left-handed superhelix around the DNA. The structure of the specific repressor–operator complex described below is consistent with this proposal. Each repressor molecule interacts directly with the bases through a pair of antiparallel β-strands inserted into the major groove of B-form DNA. The structure of the complex also shows evidence for recognition by the repressor of sequence-dependent distortions or flexibility of the phosphodiester backbone, which could mediate sequence specificity where the base pairs themselves are inaccessible. Studies of operator binding *in vitro* and repression efficiency *in vivo* indicate that the structure observed in the crystal represents the specific repression complex in solution[23].

## Structure determination

The *met* repressor protein was overexpressed and purified as before[24]. A self-complementary 19-base oligonucleotide, with sequence 5′-TTAGACGTCTAGACGTCTA-3′ (that is, two tandem consensus *met* boxes flanked by T·A base pairs and one unpaired 5′-T), was synthesized on an Applied Biosystems 381A oligonucleotide synthesizer, purified by anion exchange and reversed-phase high-performance liquid chromatography (HPLC), and annealed by heating to 80 °C for 5 min, followed by slow cooling to room temperature, for use in crystallization trials. Oligonucleotide fragments recovered from these trials are bound specifically by repressor in gel retardation assays (I. Manfield, personal communication). Crystals were grown by hanging-drop vapour diffusion over wells containing 10 mM sodium cacodylate buffer, pH 7.0, 1 mM sodium azide and 30–35% (v/v) freshly distilled 2-methyl-2,4-pentanediol. The drops contained equal volumes of well solution and solutions containing 5 mg ml$^{-1}$ oligonucleotide, 6–12 mg ml$^{-1}$ *met* repressor, 15–30 mM calcium chloride, 10 mM sodium cacodylate buffer, pH 7.0, 6 mM sodium chloride and 1 mg ml$^{-1}$ SAM (*p*-toluenesulphonate salt; Sigma). Crystals grew in space group

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TABLE 1 Crystallographic data collection and refinement statistics

| Resolution shells (Å) | Total | $N_{obs}$ Unique | Completeness (%) | $R_{sym}$* | R† |
|---|---|---|---|---|---|
| 10.0–6.36 | 2,263 | 619 | 96.7 | 0.034 | 0.208 |
| 6.36–5.04 | 3,104 | 813 | 98.0 | 0.040 | 0.224 |
| 5.04–4.30 | 3,750 | 973 | 97.9 | 0.046 | 0.183 |
| 4.30–3.81 | 4,309 | 1,101 | 98.0 | 0.060 | 0.187 |
| 3.81–3.46 | 4,732 | 1,210 | 98.2 | 0.075 | 0.196 |
| 3.46–3.19 | 5,048 | 1,308 | 98.3 | 0.114 | 0.215 |
| 3.19–2.98 | 5,131 | 1,382 | 97.6 | 0.316 | 0.301 |
| 2.98–2.80 | 5,144 | 1,450 | 96.7 | 0.657 | 0.376 |
| Totals | 33,481 | 8,856 | 97.7 | 0.074 | 0.220 |

Crystallographic statistics for data used in the refinement of the repressor–operator complex. Data were collected at room temperature on film using an Arndt-Wonacott Oscillation camera at station 9.6 of the SERC Daresbury Synchrotron Radiation Source with wavelength 0.91 Å. Two crystals were used for the complete dataset, collected as 1.8° oscillations with crystallographic *c*-axis roughly aligned along the rotation axis. Films were digitized using a Joyce–Loebl Scandig densitometer, and the images processed with the program MOSFLM[39] using the profile-fitting option. Further processing was done using the CCP4[39] suite of programs except where noted, leading to the statistics quoted in the table. The structure of the protein component was solved using molecular replacement, with the refined holorepressor structure[19] as search model. POLARRFN was used to solve the rotation function, and MERLOT[40] the translation function, the resulting model being optimized by rigid-body refinement to give $R=0.51$ to 3.5 Å resolution. The diffraction pattern shows clusters of high-intensity spots at 3.4 Å resolution, lying roughly in the *hk0* plane. This indicates that the DNA duplexes lie roughly in this plane, and that this region of the pattern would be poorly phased by calculation from the protein model alone. Accordingly, difference and Sim-weighted[41] Fourier maps phased from the protein model were calculated at 20–4 Å resolution, and inspected using the computer graphics program FRODO[42]. Difference electron density corresponding to the phosphodiester backbone of the oligonucleotide was visible near the surface of the repressor molecules bearing the antiparallel β-ribbons. An idealized B-form DNA duplex, corresponding to the central 16 base pairs of the oligonucleotide, with helix order 10.5, was fitted to this density, with its central axis of 2-fold symmetry coincident with a crystallographic dyad. Refinement was initially with the minimization option of X-PLOR[43], and subsequently with the TNT[44] package, with restrained individual B factors allowed to vary in the later cycles. All residues of both the protein and DNA components were located in Fourier maps, and included in the refinement, together with 36 ordered water molecules, giving 2,166 nonhydrogen atoms in the final model. 250 refinement cycles were done, leading to a final *R* factor of 0.220 for all data in the resolution range 10–2.8 Å, and 0.197 for data with $F_{obs} > 3\sigma(F_{obs})$. The r.m.s. deviations from ideal geometry are 0.018 Å for bond lengths, 1.68° for bond angles, 0.007 Å for planar groups and 6.6 Å$^2$ for B factors of bonded atoms. No residues in the final model deviate significantly from the allowed areas of the Ramachandran plot. Coordinates have been deposited in the Brookhaven Protein Data Bank.

* $R_{sym}=\sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i |I(h)_i|$, where *h* are unique reflection indices.

† Crystallographic *R* factor: $R=\sum_h ||F_{obs}| - |F_{calc}|| / \sum_h |F_{obs}|$.

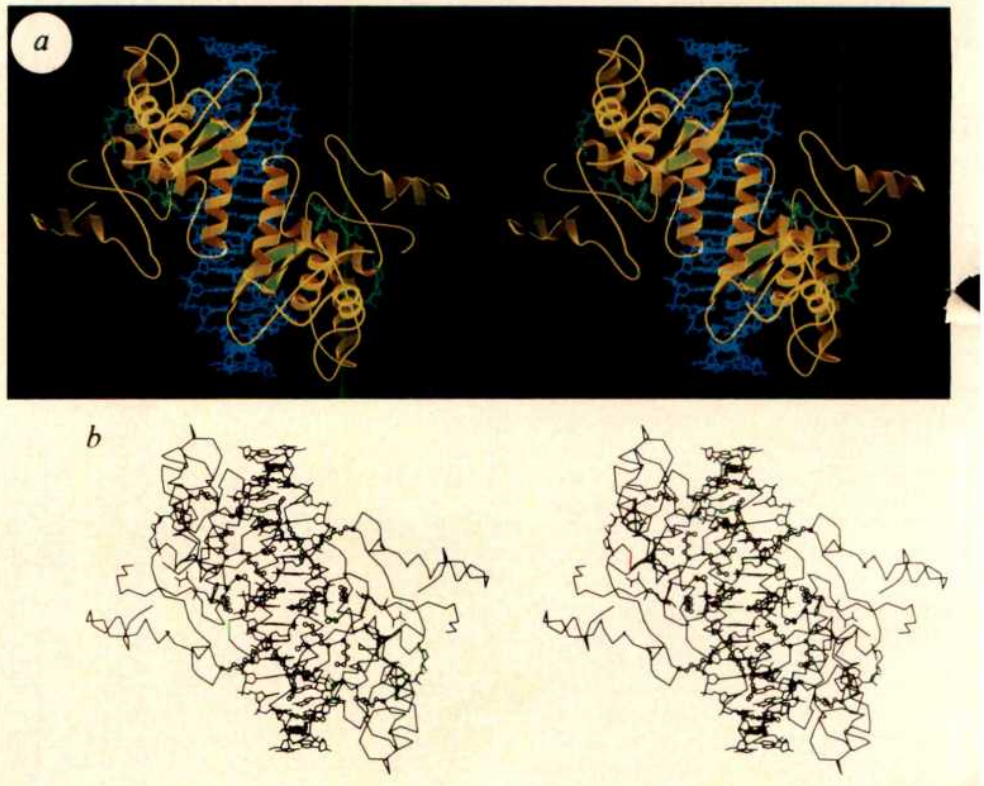


FIG. 1 *a*, Stereo view of the overall structure of the complex, with the two repressor dimers, one at the upper left and the other lower right, shown as ribbon representations[45,46]. The DNA and SAM molecules are shown as ball-and-stick models. The repressors are coloured gold, with the faces of their β-strands pale green, the DNA blue and the SAM molecules green. The view is along a crystallographic 2-fold axis that passes through the centre of the DNA and relates the two repressor dimers, and the two halves of the oligonucleotide. The repressor antiparallel β-ribbons occupy the major groove of the DNA, whereas the A helices (see Fig. 2 for helix labelling) of adjacent dimers form a long, antiparallel protein–protein contact above the minor groove in the centre of the diagram. The SAM molecules lie on the outer surface of the complex, distant from the DNA. *b*, Similar view to *a* but with repressors shown as α-carbon traces, and side chains that contact the DNA as ball and stick. The only residues that interact directly with the bases in the major groove are Lys 23 and Thr 25 from each of the four subunits, which lie on the β-strands. The other side chains shown are in contact with the phosphate backbone.

$P6_222$ as hexagonal rods, up to 0.7 mm in length. The unit cell parameters are $a=b=121.35$, $c=84.81$ Å, with one repressor dimer and one oligonucleotide strand (that is, half a duplex) in the asymmetric unit, corresponding to a solvent content of 58%. The diffraction patterns of fresh crystals extend to 2.6 Å resolution, but radiation damage rapidly reduces their quality. Intensity data were collected on oscillation films and processed to a final dataset with a merging *R* factor of 0.074 for 50–2.8 Å resolution. The structure was solved by molecular replacement and refined to a crytallographic *R* factor of 0.22. Crystallographic details are shown in Table 1.

## Overall structure of the complex

The structure of the complex (Fig. 1) shows two repressor dimers bound to a single duplex oligonucleotide, with the noncrystallographic 2-fold axes of the dimers roughly coincident with local DNA 2-fold axes passing through the centres of *met* box sequences, between base pairs 4 and 5, and 12 and 13, respectively (see legend to Fig. 4 for DNA numbering scheme). An exact crystallographic 2-fold axis passes through the centre of the complex between base pairs 8 and 9, relating the two dimers and the strands of the DNA. The ends of the oligonucleotide interact with adjacent duplexes in the crystal lattice to form pseudocontinuous helices. The unpaired 5′T(−1) lies in the major groove of the next duplex, where it makes a Hoogsteen base pair with A17 to form a T·A·T triple helical interaction. Similar interactions have been observed for G·C·G and I·A·T in the lattices of the CAP and glucocorticoid receptor complexes, respectively[13,17].

Each repressor binds with its double-stranded antiparallel

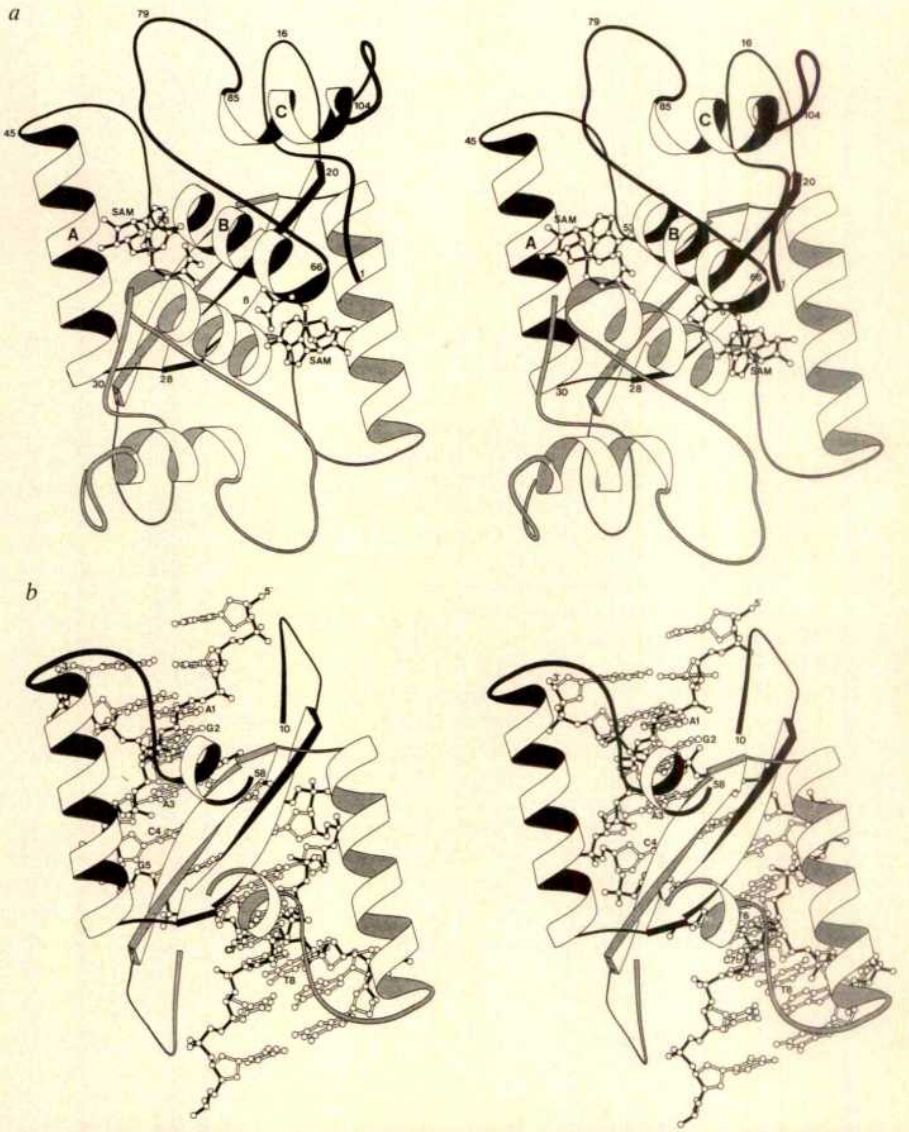


FIG. 2 *a*, Stereo diagram of a ribbon representation of the holorepressor structure determined in the absence of operator[19], viewed along the molecular 2-fold axis. One subunit is darkly shaded, with the three α-helices (A, B and C) and the β-strand labelled. Some residue numbers are indicated. The other subunit is lightly shaded. Ball and stick SAM molecules lie on the upper surface, with the positively charged sulphur atoms marked with a plus sign. The β-strands, one from each subunit, that form the antiparallel β-ribbon lie on the far side of the repressor. Residues 12–20 form flexible loops immediately preceding each β-strand. *b*, Stereo diagram of part of the repressor–operator complex in the same orientation as *a*. Only residues 10–58 of the repressor are shown, together with the part of the operator in contact with it. The DNA is shown as a ball-and-stick model, with filled bonds for the sugar–phosphate backbone, and open bonds for the remainder of the ribose rings and the bases. The central region of the DNA shown corresponds to one *met* box, and the bases have been labelled along one strand. The non-crystallographic 2-fold axis of the repressor coincides with the central 2-fold of the *met* box. The β-ribbon lies in the major groove, which faces the reader, whereas the minor groove lies to the upper left and lower right. *c*, Detailed view of the β-ribbon residues 21–29 (yellow) lying in the major groove of the DNA (blue), in a similar orientation to *b*. Hydrogen-bonds are shown as dotted lines. The side chains of Lys 23 and Thr 25 from each strand interact with the bases G2 (and G10′) and A3 (and A11′).

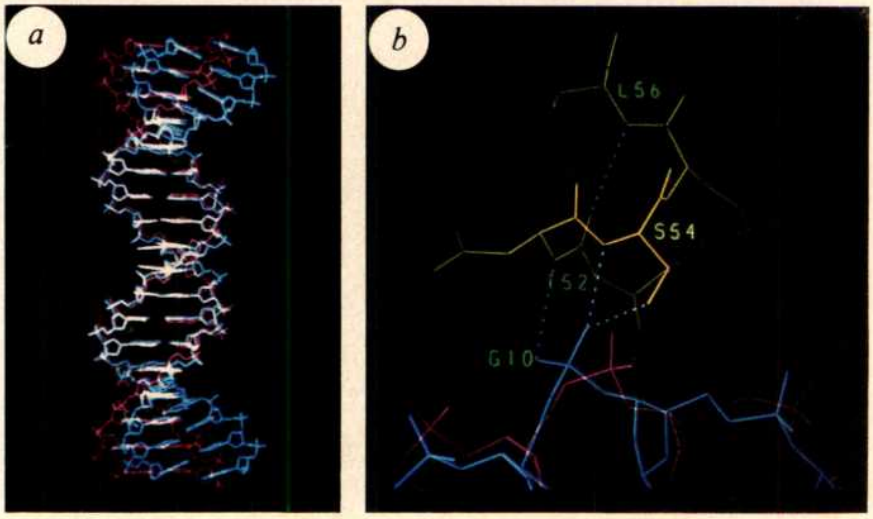


FIG. 3 *a*, DNA in the complex (blue) superimposed on idealized 10.66-fold B DNA (red). The view is perpendicular to that shown in Fig. 1, with the central crystallographic 2-fold axis in the plane of the paper. The repressors, if shown, would lie in the major groove at the upper right, slightly in front of the DNA, and lower right, slightly behind. The central 10 base pairs superimpose well. The 25° bends centred on the repressor sites correspond to compression of the major groove around the bound proteins. In the central region, the sugar-phosphate backbones superimpose well, except on the right-hand side near the centre. This corresponds to G10 and G10′ and their 5′ phosphates. *b*, View of phosphate G10 and its interaction with the N terminus of the repressor B helix. DNA colouring as in *a*, with the protein yellow. Phosphate oxygens accept hydrogen-bonds from main-chain amides of Asn 53 and Ser 54, and the side-chain hydroxyl of Ser 54. The position of the equivalent phosphate group in idealized B DNA is displaced, and could not accept these hydrogen-bonds.

β-ribbon inserted into the major groove of the DNA (Fig. 2). Side chains Lys 23 and Thr 25 on the ribbon surface of each subunit make direct hydrogen bonds to the edges of base pairs (Figs 1*b* and 2*c*), making the major contribution to sequence specificity in the complex. There are also numerous contacts to the phosphate backbone, mainly from the amino termini of the B helices and the flexible loops at residues 12–20. The two repressor dimers interact with each other, forming a tight contact between their A helices, across the crystallographic 2-fold axis (Fig. 1*a*). This contact would account for the observed cooperativity of binding of multiple repressors to extended tandem repeats of *met* box sequences in natural operators[21].

The SAM molecules bind to the faces of the repressors, remote from the DNA, with their positively charged sulphur atoms lying at the carboxy termini of the B helices, as in the holorepressor structure[19]. Corepressor binding does not cause significant structural change in the protein, though it greatly increases the affinity for the operator. *S*-adenosylhomocysteine, a SAM analogue lacking the *S*-methyl group, and with a neutral rather than positively charged sulphur atom, binds to the repressor but does not affect its affinity for the operator (T. McNally, personal communication).

## Structure of the repressor

The overall structure of the repressor when bound to DNA is little changed from that of the holorepressor (Fig. 2*a*), or indeed of the free aporepressor[19]. The repressor has a relatively rigid core, but two flexible loops, residues 12–20 and 78–83, have high-temperature factors in the absence of DNA, and adopt different conformations in different crystal environments. When bound to DNA, the 12–20 loop has a completely different conformation from the β-hairpin it adopts in the holorepressor, and wraps around the phosphate backbone (Fig. 2*b*). Glycine 15 has a positive φ angle in both conformations, as residue 2 of a type II′ turn in the holorepressor, and residue 3 of a type II turn in the complex, allowing it to donate a hydrogen-bond to a phosphate oxygen in the latter. Apart from the two N-terminal residues of each protein monomer, the entire protein and DNA chains are well ordered in the complex.

## Operator conformation

The DNA in the complex is B form, with the central 10 base pairs as a straight 10.66-fold helix. This is flanked by bends in the helix axis of about 25° towards the major groove (Fig. 3*a*). The bends lie at the centres of the *met* boxes, and correspond

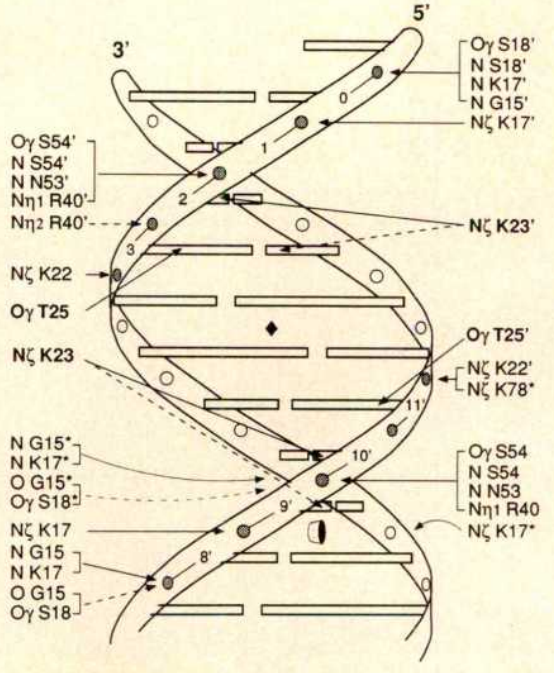


FIG. 4 Schematic diagram of protein–DNA contacts for one half of the complex, in a similar orientation to Fig. 2. The local 2-fold axis (represented by the diamond) passes through one repressor dimer and the centre of the first *met* box. The symbol below base pair 9′ represents the crystallographic dyad viewed obliquely. Phosphate positions on the DNA-ribbon backbone are shown as circles, and the bases as horizontal bars. Solid and dashed arrows indicate direct and water-mediated protein–DNA hydrogen-bonds, respectively. Curved arrows show contacts to phosphates on the back surface of the DNA. Bold characters signify residues making direct hydrogen-bonds to the bases. The two subunits of the repressor dimer are numbered 1–104 and 1′–104′, whereas residues from the symmetry-related dimer are marked with a star. The numbering scheme for the top strand of the oligonucleotide is shown below. The bottom strand is numbered in the same way from the 5′ end, but with primes on the numbers. As the crystallographic 2-fold axis passes between base pairs 8 and 9 in the complex, any base *n*′ is symmetry-related to base *n*, and has an identical environment. Bases 1–8 (bold type) and 9–16 correspond to *met* boxes.

```
   −1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
5′-  T T A G A C G T C T A G A C G T C T A -3′
3′- A T C T G C A G A T C T G C A G A T T -5′
```

to compression of the major groove to a width of 9.4 Å around the bound repressor $\beta$-ribbons, and opening of the opposite minor groove to 8.3 Å. Major and minor groove widths would be 11.7 and 5.7 Å, respectively, for ideal B-form DNA[25]. The bends and groove width variations are essential to the complementarity of fit to the repressor surfaces, and to establishment of the tight protein–protein contact.

Roll and tilt angles for the bases in the central region are low, remaining within 3° and 4° of zero, respectively. Bending is accompanied by an increase of these parameters to about 5° at the *met* box centres, and higher values towards the ends of the duplex. Propeller twist angles are also low, averaging −8.8°, but peak at −17° for base pair A3, which is also associated with the bend. Ribose ring puckers are not well defined at this resolution, and were not specifically restrained during refinement, but deviate little from their original C2′-*endo* conformation in the initial model. Although phosphate positions are well defined, the backbone torsion angles are not, and little dependable information can be derived from the latter. The helical twist angle between base pairs averages 34.1° overall, giving a helix order of 10.6, corresponding closely to that of random sequence DNA in solution[26]. Deviations from this average are small, with one important exception. The central T8–A9 step is overwound to 44°, whereas the flanking C–T and A–G steps are underwound

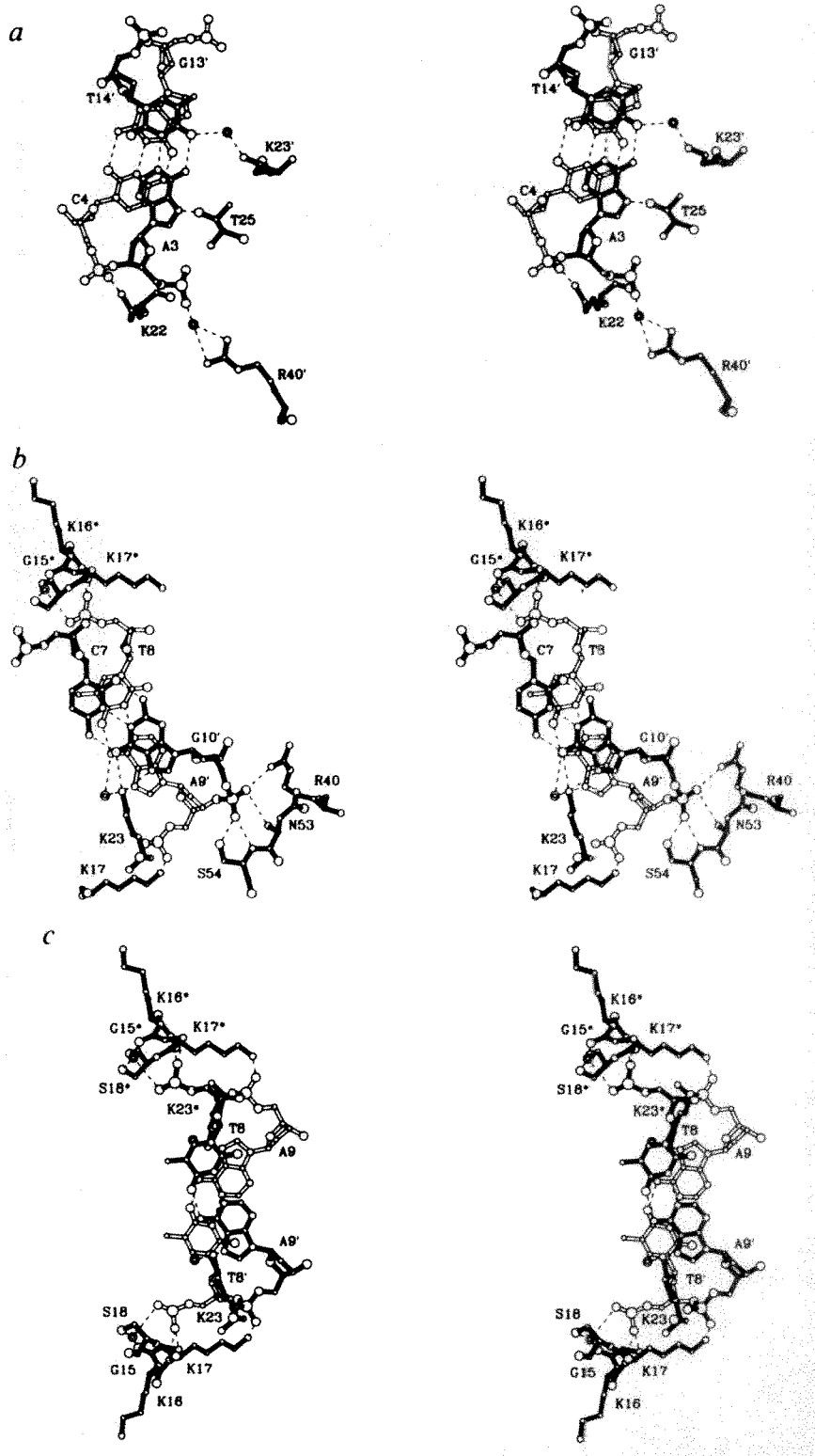


FIG. 5 Stereo diagrams of some of the base pairs, showing important interactions with the repressor, viewed along the DNA helix axis. Each base pair is shown with filled bonds, and the following base pair with open ones. Protein residues in contact are also shown, with hydrogen bonds as dotted lines. Double circles indicate ordered water molecules. *a*, Base pair A3·T14′, and its contact to Thr 25. *b*, Base pair C7·G10′, with contacts to Lys 23. Also shown is the contact from the phosphate to Asn 53 and Ser 54 of the B helix. *c*, The central T–A dinucleotide step showing the interactions of the phosphate backbone with the flexible 12–20 loop. The lack of base stacking at this step is clear in this view.

to 28°. This is associated with the anomalous position of the G10 5′ phosphate, which is displaced by 2 Å from its expected position in regular B-form DNA (Fig. 3*b*). This displacement allows a strong interaction with the N terminus of the repressor B helix. The conformation of the central CTAG sequence is reminiscent of the alternating B-form DNA structure proposed for polyd(A-T)[27], where alternating T-A and A-T dinucleotide steps are successively over- and underwound, respectively, as a result of weak stacking interactions in the T-A steps. In the absence of a three-dimensional structure for the free operator, it is impossible to tell how much of the twist variation and backbone movement is due to sequence-dependent conformation, as opposed to distortion induced by protein binding or crystal packing forces. T-A steps in the structure of the dodecamer CGCATATATGCG[28] show phosphate positions intermediate between those in our complex and regular B-form DNA, whereas that of the B DNA decamer CGATTAATCG[29] shows low twist at the central T-A, emphasizing its deformability rather than inherent high-twist nature.

## Repressor–operator interactions

The high affinity of the repressor-operator complex arises from exclusion of solvent and ions from the interface, and formation of a large number of hydrogen-bond and salt-bridge interactions. Computer graphics docking of a single repressor dimer to ideal B-form DNA in the correct orientation results in a loss of static solvent-accessible surface[30] of 208 Å$^2$, but the bending of the DNA in the observed structure of the complex raises this to 648 Å$^2$. Binding of two repressors, with extensive solvent exclusion in the protein-protein contact, raises the total to 1,732 Å$^2$ for the whole complex. This is in the range expected for similar-sized macromolecular complexes, but less than the 2,900 Å$^2$ observed for the *trp* repressor-operator complex[12].

Direct contacts to the DNA originate mainly from three regions of the protein, and are summarized in Fig. 4, with detailed interactions shown in Fig. 5. The first region includes the only direct contacts to the bases, made by side chains of the $\beta$-strands lying in the major groove (Figs 1*b* and 2*c*). The hydroxyl groups of Thr 25 and Thr 25′ donate hydrogen-bonds to N7 of A3 and A11′, respectively. The Lys 23 side chain hydrogen-bonds to O6 and N7 of G10′, whereas Lys 23′ only bonds to O6 of G2, reflecting a slight departure from the local 2-fold symmetry. Both lysines also make water-mediated hydrogen-bonds to adjacent base pairs, to N7 of A9′ and O4 of T14′, respectively. The van der Waals surface of the $\beta$-strands makes a loose fit to the major groove, leaving room for a number of solvent molecules to intrude. Only four ordered solvent molecules are visible at this resolution, sandwiched between the protein and the major groove, but there must be others filling the remaining volume.

The second major contact region is the N terminus of the B helix, which interacts strongly with the 5′ phosphate of G10′. This is similar to the commonly observed phosphate-binding sites at helix termini in other proteins and, in particular, other repressor-operator complexes[6,9]. There are hydrogen bonds from the main-chain amides of Asn 53 and Ser 54, and the side-chain hydroxyl group of the latter (Fig. 3*b*). As described above, this phosphate group lies in an anomalous position as a result of the base-stacking properties of the CTAG sequence, and the interaction can therefore be regarded as sequence-dependent. An identical interaction occurs between the end of the B′ helix of the other subunit and phosphate G2.

The third major region of protein-DNA interactions is the flexible loop, residues 12-20. The main-chain amides of Gly 15 and Lys 17 interact directly with the phosphate of T8′, and the side-chain hydroxyl of Ser 18 makes a water-mediated contact. This phosphate lies directly across the DNA duplex from the one interacting with the B helix of the adjacent repressor. The other subunit of the repressor makes similar, but more extensive, contacts to the phosphate of T0.

In addition to these interactions, there are a number of other side-chain contacts to phosphate groups, including Lys 17 and 17′ from the flexible loop, Lys 78 from an adjacent loop and Lys 22 and 22′ at the ends of the $\beta$-ribbon. Arginine 40 and 40′ extend from the A helices towards the minor groove, and contact phosphates G10′ and G2. The complete complex has eight direct hydrogen bonds from the two protein dimers to base pairs, and 38 to phosphates.

## Cooperative protein–protein interactions

The cooperative interaction between adjacent repressors is mediated by an extensive hydrophobic contact between the crystallographically related antiparallel A helices, interspersed with a network of water-mediated hydrogen-bonds. Hydrophobic interactions predominate near the ends of the helices, with a lattice of hydrogen-bonds in the centre linking Thr 37 to Gln 44*, and Arg 40 to Arg 40* by water molecules. One water lies on the crystallographic 2-fold axis, making four tetrahedrally arranged bonds to Thr 41 and 41*, and Asp 38 and 38*.

## Discussion

The validity of the crystal structure as a model for the repression complex has been tested by site-directed mutagenesis of both repressor and operator[21,23]. For instance, mutations of residues in direct contact with bases, such as Lys 23 (to Glu, Ala or Arg) or Thr 25 (to Val or Gln) result in large losses of affinity *in vitro*, and repression efficiency *in vivo*, whereas mutations on the outer surface of the complex have little effect. The mutation Thr 37 to Ala results in large reductions in affinity and repression efficiency, presumably owing to loss of cooperativity resulting from destabilization of the protein-protein contact between repressors. Systematic mutation of each base in a double *met* box operator also yields consistent results (T. McNally, personal communication). The largest reductions in affinity result from mutation of G2 or A3, and their symmetry equivalents, which form direct hydrogen bonds to the protein. It is also noteworthy that A3, and the related T6, are the most conserved bases in the natural operators[21]. The phosphates interacting with the B helix termini in the crystal structure correspond exactly to those whose ethylation most strongly interferes with complex formation in solution[21].

There are two broad principles of sequence-specific recognition in protein-DNA complexes, and Otwinowski *et al.*[12] coined useful terms to describe them: 'direct readout' arises from complementary hydrogen bonding between protein groups and the DNA bases exposed in the grooves, as suggested by Seeman *et al.*[31], and 'indirect readout' arises from detection of the sequence-dependent conformational variation or flexibility of the DNA itself. Each *met* repressor dimer recognizes a *met* box by direct interactions at base pairs G2, A3, T6 and C7 through Thr and Lys side chains. There are no specific hydrophobic contacts to thymine methyl groups such as those observed in other complexes, but there are sites in the operator where a mutation to thymine could not be accommodated owing to steric hindrance. There is evidence of indirect readout of the CTAG sequence across the central boundary between *met* boxes, which is due to the overwound T-A dinucleotide step, and the binding of the displaced phosphate group by helix B. Mutation of the central T-A step to A-T or G-C results in 76-fold loss of affinity in gel retardation assays (T. McNally, personal communication), despite the lack of direct contacts between the repressor and these base pairs. This interaction mediates recognition of A1 and T8 of the *met* box, but probably only in the presence of adjacent *met* boxes with bound repressors. The sequence CTAG also occurs in the *trp* repressor-operator complex[12], where the T-A step is not overwound but has high positive roll. The central bases of the *met* box, C4 and G5 make no direct contacts to the protein, but form the centre of the 25° bend in the DNA, which allows it to wrap tightly round the repressor. They have positive roll angles, which favour bending into the major groove,

as predicted for C·G base pairs by Drew and Travers[32], and may therefore contribute to indirect readout. Mutation of these bases results in moderate losses of affinity (T. McNally, personal communication). The importance of DNA conformation in the recognition of *met* boxes is also implied by the sequences of the natural operators where, although the base conservation at some positions is low, the sequence of purines and pyrimidines is highly conserved[21].

A major contribution to specific affinity in the system originates from the cooperative effect of two (or more) repressor dimers binding to adjacent sites, forming protein–protein contacts. The minimum specific operator complex consists of a dimer of repressor dimers bound to the DNA, as observed in the crystal structure. Single *met* box sites have very low affinity[21]. The Arc repressor of bacteriophage P22 has been shown by NMR spectroscopy to be structurally similar to residues 15–72 of *met* repressor, the DNA-binding region[33]. Studies of the binding of Arc to its operator[34] are consistent with formation of a similar dimer of repressor dimers, and also show high cooperativity. Mnt repressor of phage P22 is homologous to Arc, and also binds its operator as a tetramer[35]. The three repressors belong to a family of proteins bearing the same DNA-binding motif, the TraY protein from *E. coli* F episome probably being a fourth[36]. We describe this motif as a 'ribbon-helix-helix', corresponding roughly to the portion of *met* repressor shown in Fig. 2*b*. Its essential components are a two-stranded antiparallel $\beta$-ribbon that lies in the DNA major groove, two outer A helices available to make cooperative interactions with adjacent proteins along the DNA, and two inner B helices forming the subunit interface. The B helices also serve to lock the motif down onto the phosphate backbone by their N termini. Note that the complementarity of $\beta$-ribbons and nucleic acid duplexes has been demonstrated by model building[37,38]. Sequence specificity in these complexes is the result of overall complementarity between each repressor and its site, and between adjacent repressors bound to neighbouring sites at specific spacings. This is analogous to complexes involved in transcriptional activation, when several proteins bind to the DNA and interact with each other to form higher-order complexes. The *met* repressor may serve as a useful model for such systems. □

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# Large-scale structure in a universe with mixed hot and cold dark matter

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**A UNIVERSE whose density is dominated by cold dark matter (CDM) has been considered the standard model for large-scale structure formation[1], but it has had difficulty in matching the relatively quiet velocity field of galaxies[2] and the observed structure on very large scales[3]. By contrast, models with a mixture of CDM and hot dark matter (HDM) have more power on large scales[4–9], and seem more able to fit the excess large-scale power seen in galaxy surveys[3] and the microwave background fluctuations recently measured by COBE[10,11]. Using high-resolution numerical simulations, we examine the formation of structure in a mixed dark matter model containing 70% CDM and 30% HDM, the latter in the form of massive neutrinos. This model behaves like a CDM model in which the biasing factor (the relative magnitude of structure in the dark and visible components) varies from 2.5 on small scales to <1 on large scales, and can provide a consistent explanation of both the shape of the observed fluctuation spectrum and the difference in estimates of the cosmic density, Ω, on small and large scales.**

The amplitude of the fluctuations reported by the COBE collaboration[10] is close to that predicted by several theories of large-scale structure in the Universe[4,11], including what is perhaps the simplest model consistent with inflation, an unbiased CDM model. But unbiased CDM has serious difficulties in describing small-scale structure[2,12], chiefly because the depths of the potential wells on megaparsec scales lead to velocity fields larger than those observed in the galaxy distribution. To avoid this problem, the concept of bias in the galaxy distribution was introduced. Roughly speaking, one can define a bias parameter as $b = \sigma_g/\sigma_\rho$, where $\sigma_g$ and $\sigma_\rho$ are the root-mean-square fluctuation amplitudes of the galaxies and mass density, respectively, on a given scale. Davis *et al.*[2,13] showed that a CDM model with $b = 2.5$ and $\Omega = 1$ leads to a good match for a variety of galaxy-

scale measures of structure. Because galaxies are peaks in the mass distribution, some biasing is expected[14], although this 'natural bias' today should be weak on these scales[15]. The physical mechanism behind large bias is unclear at best.

On larger scales, high-bias models provide insufficient power to describe the large-scale velocity fields, the Automated Plate Measurement (APM) angular correlations or the amplitude of the COBE fluctuations. One may increase the ratio of large- to small-scale power by assuming a scale dependence of the bias, but if galaxies are rare peaks drawn from an initially gaussian mass distribution, the scale dependence should be weak[14]. To force the models to fit the APM observations, it has been suggested[16,17] that bias is modulated by large-scale processes (such as a nearby quasar flooding its surroundings with ultraviolet radiation). If luminous galaxies are strongly biased relative to the mass distribution, however, there should be a strong differential clustering amplitude between faint and bright galaxies, which is not observed[18]. This fact argues for a lower $b$ value for the luminous galaxies, in which case the modulation of the bias will be less effective. Additionally, some simulations attempting to trace galaxies as they cluster find a significant bias in the velocities of galaxies relative to the dark matter, suggesting that an $\Omega = 1$ CDM universe might be consistent with a mildly anti-biased model[19] ($b < 1$). Overall, suggestions that galaxies are a biased tracer of the mass have met with mixed success and are becoming increasingly complex in an attempt to fit all the observational data simultaneously.

All models of large-scale structure must address the fact that the density of the Universe, when measured on scales of 1 Mpc or less, is consistent with $\Omega = 0.1$, and not with $\Omega = 1$ (refs 20, 21). On larger scales, observation is more difficult, but recent comparison of velocity flows with the gravitational field derived from IRAS selected galaxy samples[22-24] seems to give the opposite result: consistency with $\Omega = 1$, but not with $\Omega = 0.1$. Low values of the total mass density are also inconsistent with the COBE fluctuations. It is important to recall that estimates of density are insensitive to the fraction of the total mass density that is smoothly distributed on the scale in question, and that one makes the implicit assumption that the galaxies in the study are fair tracers of the underlying mass distribution.

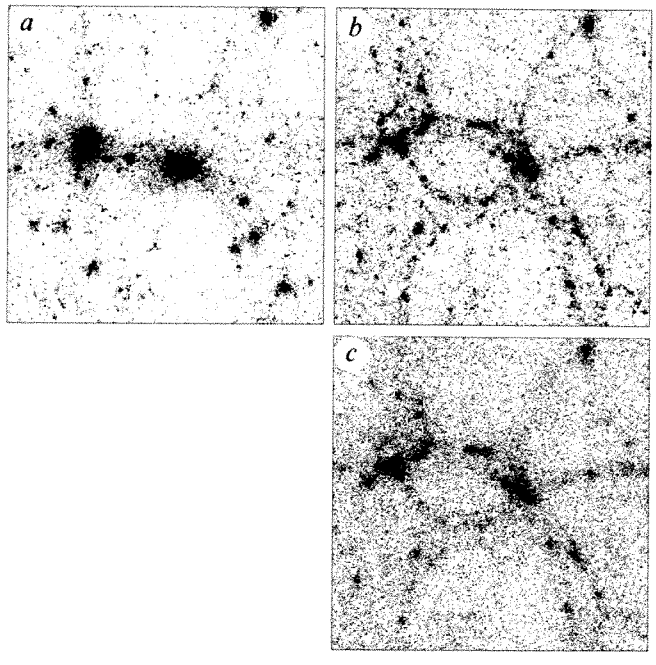


FIG. 1 *a*, Mass distribution of a pure CDM simulation of $32^3$ particles in a box of size 14 Mpc. *b*, Distribution of the cold component in a MDM simulation with the same initial conditions as the CDM model but lower initial amplitude. *c*, Distribution of the hot component in the MDM simulation. The HDM is much smoother and clusters around the larger CDM concentrations, although with considerably less density contrast.

An alternative possibility still consistent with inflation is for some fraction of the mass density of the Universe to be more smoothly distributed than the galaxy distribution. The ultimate smooth background would be a cosmological constant ($\Lambda$), and Efstathiou *et al.*[25] have suggested a model with smooth fraction $\Omega_\Lambda = 0.8$, $\Omega_{\rm total} = 1$. This model provides a good fit to the angular correlations in the APM data, reasonable large-scale flows, consistency with COBE fluctuations and appropriate galaxy velocities on small scales. Apart from the lack of justification for introducing a vacuum energy density that precisely at this moment is becoming dominant in the Universe, a cosmology dominated by a smooth background is incapable of explaining the high $\Omega$ estimates derived from the IRAS studies.

A mixed dark matter (MDM) cosmology, on the other hand, introduces no more parameters than the $\Lambda$ model and removes the special timing of such a model. The behaviour of the HDM component is sufficiently different from the CDM fraction to allow interesting scale-dependent processes to occur. Such models have been investigated sporadically[5-9], but have been criticized because the assumption that two types of dark matter were present seemed too complex. It is fair to say, however, that observations now rule out the simplest models, and that more complicated models must be considered on their merits.

The additional physical principles introduced with HDM cosmologies are particle free streaming and retardation of growing modes[26,27]. If we consider a single species of neutrino and assume the usual thermal history, the present mass density contributed by a neutrino of mass $m_\nu$ (in eV) would be $\Omega_\nu = m_\nu/(93h^2)$. Such a particle would become nonrelativistic at an epoch $(1+z)_{\rm nr} = 18{,}500(m_\nu/10\ {\rm eV})$, where $z$ is redshift. Primordial velocities then decay adiabatically with a characteristic velocity of

$$v_{\rm char} = \frac{3kT_\nu(t)}{m_\nu c} = 15(1+z)\left(\frac{10\ {\rm eV}}{m_\nu}\right)\ {\rm km\ s^{-1}}$$

where $kT_\nu$ is the thermal energy of the neutrons. At earlier epochs, the free streaming of the relativistic neutrinos erased all their initial fluctuations on scales smaller than the comoving horizon scale, although the fluctuations of the CDM component remain. After the epoch of matter dominance, $(1+z)_{\rm m} = 6{,}900$ ($\Omega = 1$, $h = 0.5$), fluctuations in the cold component are gravitationally unstable on all scales. But the neutrino component is thermally too hot to cluster on the smallest scales and, roughly speaking, is Jeans unstable only for comoving wavenumbers

$$k^2 < k_{\rm J}^2 = \frac{4\pi G\rho}{(1+z)^2 S_{\rm A}^2}$$

where $S_{\rm A}$ is the adiabatic sound speed and $\rho$ is the mass density. As the neutrinos are collisionless, $S_{\rm A}$ is not strictly defined, but for purposes of the Jeans stability[27], $S_{\rm A} \approx v_{\rm char}$. Thus we have $k_{\rm J} = 8h(1+z)^{-1/2}(m_\nu/10\ {\rm eV})\ {\rm Mpc}^{-1}$, so that galaxy-sized fluctuations have only recently become Jeans unstable to the neutrino component.

On large scales, where both components are gravitationally unstable, the amplitude of linear fluctuations can grow as $a^\alpha$, with $\alpha = 1$ for an Einstein–de Sitter universe. On scales where the neutrino component is stable, the linear growth is retarded with an effective rate $\alpha_{\rm s} = \frac{1}{4}[(24f+1)^{1/2} - 1]$, where $f$ is the fractional mass density that is Jeans unstable[26]. As an example, we consider a model with $\Omega_\nu = 0.3$, cold dark matter density $\Omega_c = 0.7$ and $h = 0.5$, implying $m_\nu = 7.0$ eV and $\alpha_{\rm s} = 0.805$. For scales that have remained Jeans stable to the neutrinos up to the present epoch, the cumulative linear growth is reduced by a factor $(1+z)_m^{1-\alpha_{\rm s}} = 5.6$. This is the factor by which the transfer functions at high frequency will differ when a pure CDM universe is compared with a MDM universe having the same initial amplitude. Thus the growth of small-scale structure can be

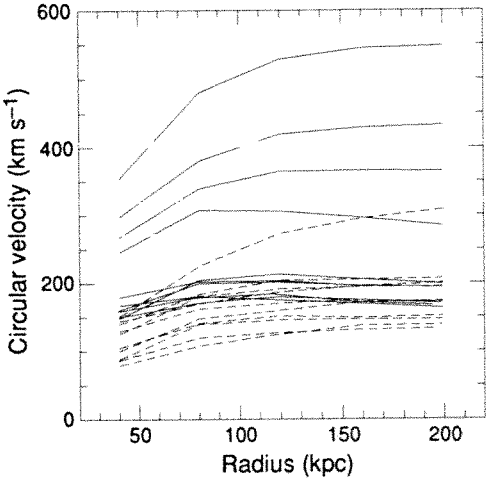


FIG. 2 Circular rotation curves $(GM(r)/r)^{1/2}$ for the 10 largest haloes in the CDM (solid curves) and MDM (dashed curves) simulations. This CDM model, with $\sigma(8h^{-1})=0.9$, produces haloes that are too deep to be associated with individual $L_*$ galaxies, whereas the MDM haloes have very flat rotation curves of an appropriate amplitude.

substantially slowed. Note that if $\Omega_\nu \gtrsim 0.5$, the hybrid model behaves much like a pure HDM universe where pancake formation on supercluster scales initiates the nonlinear clustering phase.

Because the random kinetic energy of the final nonlinear structure is proportional to the gravitational potential, one expects the retarded small-scale growth of the MDM model to lead to lower peculiar velocities on small scales. The linear theory of the retarded growth has been fully discussed[4,6,7], but the nonlinear aspects of this problem have not previously been considered except for isolated seed accretion[28].

To demonstrate the effects of a mixed hot and cold dark matter distribution, we present the results of two simulations using a P$^3$M $N$-body code[29]. We consider a periodic box of coming size 14 Mpc, evolved from an epoch $(1+z)=20$ to the present. Our potential grid is $64^3$ and our comoving softening length is 0.2 grid cells. First we ran a pure CDM model of $32^3$ particles with initial conditions generated by a random realization of the Zeldovich approximation, with an initial amplitude chosen to yield $\sigma_\rho(8h^{-1}\,\mathrm{Mpc})=0.9$ at the present epoch. For these parameters, the particle mass is $5.8\times10^9$ solar masses ($M_\odot$). This model was compared to an $\Omega_\nu=0.3$, $\Omega_c=0.7$ simulation with equivalent initial conditions. We used $32^3$ cold particles and $32^3$ hot particles of mass $4.1\times10^9\,M_\odot$ and $1.7\times10^9\,M_\odot$ respectively. The neutrino Jeans length at $(1+z)=20$ is two-thirds the size of the box, so it is reasonable to start the neutrinos smoothly distributed on a uniform grid. We give the neutrino particles an initial, randomly oriented, velocity drawn from the primordial Fermi-Dirac distribution function

$$f(p)\propto\frac{p^2\,\mathrm{d}p}{e^p+1}$$

where

$$p=\frac{v}{c}\left(\frac{m_\nu c^2}{kT_\nu^0}\right)(1+z)^{-1}=0.20v\left(\frac{m_\nu}{10\,\mathrm{eV}}\right)(1+z)^{-1}\,\mathrm{km\,s^{-1}}$$

and $T_\nu^0=1.95$ K is the present temperature of the neutrino background. This is admittedly a crude simulation of the phase-space distribution, but fortunately the essential physics do not suffer from discreteness effects because a luminous ($L_*$) galaxy halo consists of hundreds of particles.

Up to $(1+z)=20$, the linear deficit for the Jeans-stable modes is a factor of $(6{,}900/20)^{1-\alpha_s}=3.13$. As this factor applies to essentially all spatial frequencies in our simulation, the appropriate initial conditions for the cold component in the MDM models are a scaled version of a pure CDM spectrum. Holtzman[4] provides fits to the present transfer function for both the MDM and pure CDM models. If they are to have the same fluctuation amplitude $\sigma_\rho(8h^{-1}\,\mathrm{Mpc})$ today, then the hybrid model must have an initial fluctuation amplitude 1.55 times higher than the pure CDM model (implying that its COBE-scale fluctuations will be larger by this same factor than the pure CDM model). Thus the cold component in the MDM simulation starts with the same realization as the CDM simulation, but with a fluctuation amplitude lower by a factor $(1.55/3.13)=0.495$. The initial velocity field of the cold component must also be reduced by the factor $\alpha_s$ to account for the slower growth rate.

Figure 1 shows the resulting mass distributions at $z=0$ for the two simulations. As expected, the clustering in the pure CDM model is stronger. In the hybrid simulation, the neutrino distribution is considerably smoother and shows strong clustering only around the largest haloes, consistent with the present neutrino Jeans length of 2 Mpc. The potential wells of the haloes in the mixed model are shallower than in the pure CDM model, as shown in Fig. 2 where we plot circular velocities, $v(r)=(GM(r)/r)^{1/2}$, for the ten largest haloes in each simulation. The pure CDM model has a few potentials that are too deep to be associated with normal galaxies and too abundant to be associated with large groups of galaxies, whereas the MDM model has a reasonable number of haloes that could be associated with the haloes of $L_*$ galaxies.

Another important statistics is the r.m.s. relative dispersion of pairs of cold particles. For the CDM and MDM models the pairwise velocity dispersions are 400 km s$^{-1}$ and 200 km s$^{-1}$, respectively, and are nearly constant as a function of pair separation. Both of these numbers are lower than would be measured in a representative sample of the Universe because this small-scale simulation contains no large clusters. The large velocities in CDM models have been difficult to match to observations. Here we see that the velocity field is reduced a factor of two in the MDM model, yet by design $\sigma_\rho(8h^{-1}\,\mathrm{Mpc})$ is the same for the two models.

On the scale of this simulation, the structure in the MDM model is similar to that of the high-bias CDM halo models described by Frenk *et al.*[30]. The spatial correlations of the haloes will be slightly biased relative to the mass distribution and, in combination with the reduced velocity field of the MDM model, will translate into an effective small-scale $\Omega$ which is fairly low. If these effects yield a natural bias in the galaxy distribution of $b\approx1.5$, it should be possible to explain the low $\Omega$ estimates derived from virial analysis of groups and clusters, and yet have adequate large-scale power to describe large-scale flows and the COBE fluctuations.

The MDM model thus seems to resolve a long-standing problem of large-scale structure, namely the disparate estimates of $\Omega$ on small and large scales. Velocity fields are reduced on small scales and increased on large scales, increasing the 'Mach number' of the cosmic velocity field[31]. This, combined with its other successes in matching large-scale structure in the Universe[8,32], makes the model worthy of serious consideration. Perhaps it is finely tuned, but within the range $0.2<\Omega_\nu<0.4$, the MDM model could provide a simple explanation of this cosmological puzzle. It has no more degrees of freedom than alternative models, and dispenses with the need for substantial bias in the galaxy distribution.

It has already been remarked that this MDM model provides a good fit to the fluctuations observed by COBE[9,11]. A small rest mass, of order $10^{-2}$–$10^{-3}$ eV for muon neutrinos, with a considerably smaller mass for the electron neutrino, would account for the observed deficits of solar neutrinos by means of MSW mixing[33]. A possible explanation of neutrino masses is the see-saw mechanism, which suggests 4–28 eV as a reasonable mass range for the tau neutrino[34]. Prospects for direct detection of such a neutrino are remote, but perhaps neutrino

mixing experiments, combined with current solar neutrino experiments using gallium, will eventually indicate its existence.

Our results are certainly not definitive. The volume of the simulation is too small to allow us to measure the galaxy correlations directly, but if we increase the particle mass too much, the phase-space distribution of the hot component will be too coarsely sampled. Larger simulations are now in progress.

After submitting this paper we learned of the work by Taylor and Rowan-Robinson[32] (following paper), in which a similar cosmological model is proposed as the best explanation of the COBE measurements and the existence of large-scale galactic structure. □

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# The spectrum of cosmological density fluctuations and nature of dark matter

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**COSMIC structure is thought to have arisen by the gravitational amplification of small density fluctuations in the early Universe. The evolution of fluctuations with specified magnitude and spectrum is controlled by a few fundamental parameters: the cosmic density Ω, the cosmological constant Λ, and the relative contributions of radiation and of dark and visible matter to the density of the Universe. Maps and statistical descriptions of the large-scale distribution of galaxies, from the QDOT IRAS redshift survey[1–7], along with the COBE measurements of the microwave background fluctuations[8,9] have recently transformed our understanding of large-scale structure, for which the growth of fluctuations is linear and well understood. These two sets of data effectively determine the density fluctuation spectrum in the present Universe on scales from 10 to 1,000 Mpc. Here we examine an array of structure formation models, and show that most are ruled out by the COBE and QDOT observations. We find only one completely satisfactory model, in which the Universe has density Ω = 1, with 69% in the form of cold dark matter, 30% provided by hot dark matter in the form of a stable neutrino with mass 7.5 eV, and 1% baryonic. Certain 'grand unified theories'[10,11] may provide a physical basis for such hybrid models. A Hubble constant of 50 km s$^{-1}$ Mpc$^{-1}$ is preferred to one of 100.**

On large angular scales, temperature fluctuations in the cosmic microwave background radiation (CMBR) are produced by the Sachs–Wolfe effect[12] at the end of recombination. Photons, no longer coupled to matter by Thomson scattering, climb out of the potential wells produced by the mass fluctuations, resulting in small temperature anisotropies in the surface of last scattering. The predicted correlation function of temperature inhomogeneities can be expressed in terms of the power spectrum of density fluctuations as

$$C(\theta) = \frac{1}{4\pi}\sum_l (2l+1) C_l P_l(\cos\theta) |W_l(\theta_s)|^2$$
$$C_l = \frac{8V}{\pi\tau_0^4}\int \mathrm{d}k\, k^{-2} P(k,\tau_0) j_l^2(k\tau_0) \qquad (1)$$

where $C_l$ is the angular power spectrum of temperature fluctuations and $P(k, \tau_0)$ is the power spectrum of density fluctuations at the present epoch. We assume both statistically isotropic and homogeneous perturbations. It is generally, and reasonably, assumed that the primordial density fluctuation spectrum is well approximated by a power law, $P(k) \propto k^n$ (where $k$ is the comoving wavenumber). $W_l(\theta_s)$ is a filter function representing smearing due to a finite beam size, and $\tau$ is the conformal time, $\tau = \int \mathrm{d}t\, a(t)^{-1}$, where $a(t)$ is the cosmological scale factor. In the matter-dominated regime, $\tau \approx 2H_0^2\Omega_0^{-1/2}(1+z)^{-1/2}$, for $\Lambda = 0$ (where $z$ is redshift). The Hubble radius is defined as $d_H \equiv a(t)\tau \approx 2H_0^{-1}\Omega_0^{-1/2}(1+z)^{-3/2}$. $P_l(x)$ and $j_l(x)$ are the Legendre polynomial and spherical Bessel functions of order $l$, respectively[13]. We adopt the convention $c = 1$, $h = H_0/(100\ \mathrm{km\ s^{-1}\ Mpc^{-1}})$.

Angular scales, $\theta$, on the microwave background can be related to linear scales, $\lambda_0$, at the present epoch by $\theta(\lambda_0) \approx \frac{1}{2}\Omega_0 H_0 \lambda_0$ radians, $z \gg 1$. The COBE satellite is sensitive to scales $\theta > 7°$, corresponding to fluctuations on scales $> 10^3 h^{-1}$ Mpc at the present. At recombination these scales were larger than the Hubble radius, and because microphysical processes can act only on scales less than the Hubble radius, these fluctuations must have been produced by the Sachs–Wolfe effect. The subsequent growth of these perturbations is linear, so they have retained the imprint of the primordial perturbations from their epoch of formation. In the context of inflationary scenarios this formation takes place at a time $t \approx 10^{-32}$ s (refs 14, 15). Post-recombination reionization could also produce fluctuations in the CMBR, but would lead to fluctuations larger than observational limits on small scales (but see Fukugita and Kawasaki[16] who discuss reionization in unstable neutrino models).

On smaller scales, $\lambda \ll 10^3 h^{-1}$ Mpc, the shape of the fluctuation spectra is determined by the physics of the matter and radiation components of the Universe, introducing characteristic length scales into the power-law spectrum, which should be reflected in the distribution of galaxies today. Defining the relative fluctuations in mass density as $\delta \equiv \delta\rho/\langle\rho\rangle$, where $\langle\rho\rangle$ is the mean mass density of the Universe, we can relate the variance, $\sigma_0^2$, of mass-density fluctuations on a scale $R$ to the power spectrum by Rayleigh's theorem

$$\sigma_0^2(R) \equiv \langle\delta^2\rangle = \frac{V}{2\pi^2}\int \mathrm{d}k\, k^2 P(k,\tau_0)\hat{W}_k(R)\hat{W}_k^*(R) \qquad (2)$$

where $\hat{W}_k(R)$ is the Fourier transform of a window function on a scale $R$. As it is not obvious that fluctuations in the galaxy distribution should exactly match those of the mass density field

(for example, if galaxies form at the peaks of the mass-density field they will be more correlated than the mass), we allow the variance of the two distributions to vary on large scales by the square of a bias factor, $b$, defined as[17-19]

$$\sigma_{0,g}^2(R) \equiv b^2 \sigma_0^2(R) \tag{3}$$

Finally, on intermediate scales ($\lambda \approx 100\,h^{-1}$ Mpc) the power spectrum can be probed by the large-scale velocity field generated purely by the self-gravitation of the density field. Here the variance in velocity fluctuations on a scale $R$ is related to the power spectrum by

$$\sigma_v^2(R) = \frac{(a_0 H_0 f_0)^2 V}{2\pi^2} \int \mathrm{d}k\, P(k, \tau_0)\, \hat{W}_k(R)\, \hat{W}_k^*(R) \tag{4}$$

where $f_0 \approx \Omega_0^{0.6}$ is the ratio of the rate of growth of fluctuations compared with the expansion rate. It is possible to produce velocity fields by nongravitational processes, but the observed agreement between the amplitudes of perturbations in the CMBR, galaxy and velocity fields supports the gravitational instability theory.

To place constraints on the power spectrum, and hence on fundamental parameters, we shall use data on clustering in the range $10 \lesssim \lambda \lesssim 10^3 h^{-1}$ Mpc, and constraints placed on the mass density of the Universe and linear bias parameter by the distribution of IRAS galaxies. We convert the data to a quantity related to the power spectrum at the present epoch, the fractional variance in density per ln $k$ (refs 20, 21)

$$\Delta_k^2(k) \equiv \frac{\mathrm{d}\sigma_0^2}{\mathrm{d}\ln k} = \frac{V k^3 P(k, \tau_0)}{2\pi^2} \tag{5}$$

In Fig. 1 we plot the square-root of this quantity for the QDOT redshift survey[2,3], correcting the results for redshift-space distortion[22]. We also show the variance in cells derived from cross-correlating the distribution of QDOT with its parent, the QIGC angular catalogue[6]. This requires no redshift corrections. The data cover the range $10 < k^{-1} < 70h^{-1}$ Mpc. We have not attempted to include the two-dimensional angular correlation data for the APM galaxy survey[23] or the covariance function data for radio galaxies[24] because of uncertainty about the appropriate normalization.

Similarly, we can obtain an estimate for the power spectrum on scales $\lambda \approx 100h^{-1}$ Mpc from bulk-flow studies[25]. In a spherical volume of radius $R = 40h^{-1}$ Mpc, Bertschinger *et al.*[25] find a bulk flow of $388 \pm 67$ km s$^{-1}$, and for a radius $R = 60h^{-1}$ Mpc, a bulk flow of $327 \pm 84$ km s$^{-1}$. The bulk flow is defined by

$$\mathbf{V}_B(R) = \frac{\int_V \mathrm{d}^3x\, \mathbf{v}(\mathbf{x})\, W(x/R)}{\int_V \mathrm{d}^3x\, W(x/R)} \tag{6}$$

The velocity variance on these scales, $\sigma_v^2(R)$, can be estimated by assuming that it is equal to the squared bulk flow measured on this scale, $|\mathbf{V}_B(R)|^2$. Although far from satisfactory, this is the best estimate available for perturbations on these scales.

The COBE results now allow us to constrain the very-large-scale range of fluctuations. The fractional variance in density per ln $k$ is

$$\Delta_k^2 = \frac{12}{\pi}(kH_0^{-1})^4 \Omega_0^{-1.5} C_2, \qquad k\tau_{\mathrm{rec}} \gg 1 \tag{7}$$

where $C_2$ is the angular power spectrum for $l = 2$ (equation (1)) and is related to the observed quadrupole[9] by

$$\left(\frac{\Delta T}{T}\right)_Q^2 = \left(\frac{5}{4\pi}\right) C_2 = (0.48 \pm 0.15)^2 \times 10^{-10} \tag{8}$$

As the temperature fluctuations appear the same on all scales, the spectral index on these scales is constrained to be $n = 1.1 \pm 0.5$ (ref. 9), and we shall discuss only the asymptotically scale-invariant $n = 1$ form, originally introduced to explain galaxy clustering[26,27], and later shown to be a consequence of inflationary models[14,15]. Data points corresponding to the bulk-flow and COBE results are shown in Fig. 1.

The transformation of primordial spectra from radiation to matter domination and through recombination can be described by a transfer function, $T_k$, giving the post-recombination spectrum

$$P(k, \tau) = T_k^2 P(k, \tau_i) \tag{9}$$

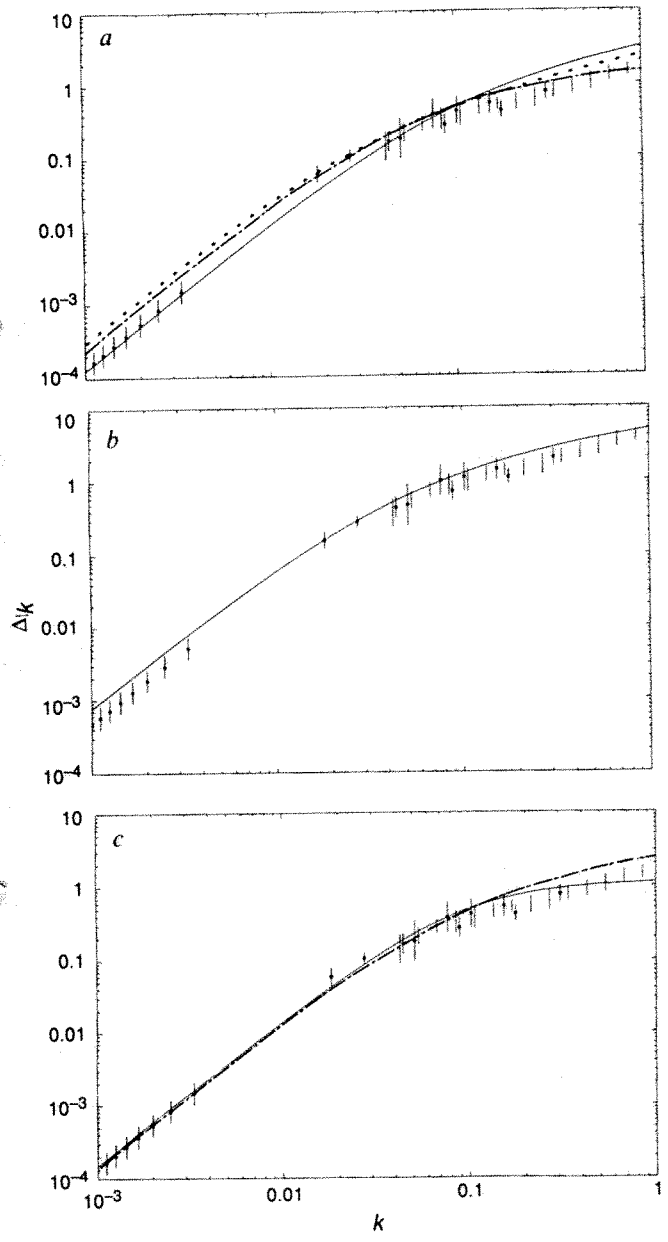


FIG. 1 *a*, Square root of the fractional variance in mass density per unit ln $k$ (equation (5)) as a function of ln $k$. All theoretical curves are normalized as described in the text. Plots *a* and *c* show only the curves for $h = \frac{1}{2}$. The data points shown are for QDOT (•, ref. 6; ●, refs 2, 3), the bulk-flow field (■) and the power spectrum extrapolated from the COBE quadrupole measurement, assuming a scale-invariant spectrum (○), with $1\sigma$ error bars. The models are: (solid line) the adiabatic CDM with $\Omega_0 = 1$, $h = \frac{1}{2}$; (dot-dashed line) the isocurvature CDM, $\Omega_0 = 1$, $h = \frac{1}{2}$ model (the CMBR points for this model are a factor of 6 smaller than those plotted for the adiabatic model); (dotted line) the model with texture plus CDM, $\Omega_0 = 1$, $h = \frac{1}{2}$. *b*, As *a*, for the adiabatic model with CDM plus vacuum energy ($\Lambda > 0$), $\Omega_{\mathrm{VAC}} = 0.8$, $\Omega_{\mathrm{CDM}} = 0.2$, $h = 1$. *c*, As *a*, for: (solid line) the CDM plus HDM model with one species of stable massive neutrino, $\Omega_0 = 1$, $\Omega_{\mathrm{CDM}} = 0.89$, $\Omega_\nu = 0.3$, $\Omega_b = 0.01$, $h = \frac{1}{2}$; (broken line) as above, but with $\Omega_{\mathrm{CDM}} = 0.89$, $\Omega_\nu = 0.1$, $\Omega_b = 0.01$, $h = \frac{1}{2}$.

where $\tau_i$ is the initial epoch when perturbations were generated. The form of $T_k$ has been given for a variety of cosmological models in refs 19, 28.

In the absence of a theoretical prediction, the amplitude of the power spectrum of perturbations is fixed by observation, conventionally by normalizing the variance of linear perturbations on a scale where optical galaxies have unit variance, $8h^{-1}$ Mpc. Equation (3) then tells us that $\sigma_0^2(8h^{-1}\,\mathrm{Mpc}) = 1/b_8^2$, where $b_8$ is the bias factor for optical galaxies on $8h^{-1}$ Mpc. But given the complexity of galaxy formation processes and small-scale dynamics, it is doubtful whether this scale is securely in the linear regime[20].

The best recent estimates of the variance of perturbations on large scales come from the QDOT redshift survey. Three dynamical tests now give similar values for the parameter $g_0 \equiv b_I\Omega^{-0.6}$, where $b_I$ is the linear bias factor for infrared selected galaxies, on a scale of $\sim 30h^{-1}$ Mpc. Dipole studies[1], least-squares fitting of predicted and observed peculiar velocities[4] and the analysis of the covariance tensor of reconstructed differential motions give $g_0 = 1.23 \pm 0.23$, $g_0 = 1.16 \pm 0.21$ and $g_0 = 1.2 \pm 0.1$, respectively. These results support the $\Omega_0 = 1$ prediction of inflationary models with very little bias. A similar result has been found by Strauss *et al.*[29,30]. The variance of perturbations on a scale of $28h^{-1}$ Mpc is then $\sigma_0^2(28h^{-1}\,\mathrm{Mpc}) = (0.21 \pm 0.07)/b_I^2$ (ref. 6), where we adopt $\Omega_0 = 1$, $b_I = 1.2$. We shall also assume that nucleosynthesis calculations imply $\Omega_b \leq 0.1$, where $\Omega_b \equiv (\rho_b/\langle\rho\rangle)\Omega_0$, and where $\rho_b$ is the mass-density of baryonic matter.

We have calculated the power spectrum at the present epoch, using COBE, QDOT and the bulk-flow measurements, and compared it with a range of cosmological models: (1) adiabatic cold dark matter (CDM), (2) adiabatic hot dark matter (HDM, for one stable massive neutrino species), (3) warm dark matter, (4) isocurvature CDM[19], (5) texture plus CDM[20,31], (6) CDM plus vacuum energy density, and (7) CDM plus HDM[28] (one stable neutrino species). Each model was tested using a Hubble parameter of $h = 0.5$ and $h = 1$, and model (7) was tested over a range of neutrino masses. The Sachs–Wolfe formula (equation (1)) for isocurvature perturbations is amplified by a factor of $\sim 6$ (ref. 32), and the CMBR fluctuations for model (5) are estimated from ref. 31.

Our criterion for assessing the models is simple. Having fixed the normalization, we require a good fit (using a $\chi^2$ test) to the combined data for $P(k, \tau_0)$. The COBE data for only two scales, the quadrupole and the r.m.s. fluctuations on $\theta = 10°$, are included. Most models are strongly ruled out by the poor $\chi^2$ fit. The models with lowest $\chi^2$ are listed in Table 1 and several are illustrated in Fig. 1.

We can immediately rule out the adiabatic HDM model as it predicts insufficient structure on small scales, even taking into account nonlinear evolution[33]. The adiabatic warm dark matter with $h = \frac{1}{2}$ fits the COBE fluctuations but predicts excessive galaxy fluctuations, requiring strong antibiasing, on small scales. The isocurvature CDM model with $h = \frac{1}{2}$ (Fig. 1*a*) is ruled out at the 99% level and exceeds the COBE fluctuations at the $5\sigma$ level (compare with ref. 32). Cosmic textures with CDM and $h = \frac{1}{2}$ (Fig. 1*a*) is also ruled out at the 99% level and exceeds the COBE fluctuations at the $2\sigma$ level.

The model with adiabatic CDM plus vacuum energy ($\Lambda > 0$), with $\Omega_{VAC} = 0.8$, $\Omega_{CDM} = 0.2$, put forward to reconcile the angular correlation excess in the APM galaxy survey[34], requires an unphysical low bias factor ($b_I = 0.46$) for consistency with the QDOT inferred velocity field data. Although an acceptable $\chi^2$ is obtained with this model ($\chi^2 = 22.35$), if $h = 1$, it exceeds the COBE fluctuations at the $1.5\sigma$ level (Fig. 1*b*). Given that there is no physical reason for either the strong antibiasing ($b < 1$) required on all scales, or for the non-zero value of $\Lambda$, we do not consider this option further (but see ref. 35 for an alternative view).

The adiabatic CDM model with $\Omega_0 = 1$, $h = \frac{1}{2}$, is also shown in Fig. 1*a*. The fit to the COBE data is excellent. Unfortunately it predicts far too much power on small scales so that a significant degree of antibiasing would be needed. Such a model has been advocated by Carlberg *et al.*[36] and Couchman and Carlberg[37], who argue that increased mergers in cluster haloes on a scale $\lambda \approx 1h^{-1}$ Mpc produced antibiasing. More seriously for this model, it does not predict high enough values for the bulk flow on scales $\sim 100h^{-1}$ Mpc, by $2\sigma$.

TABLE 1 $\chi^2$ values for models

| Model | $\chi^2$ | $P(>\chi^2)$ |
|---|---|---|
| A | 47.85 | $1.133 \times 10^{-3}$ |
| B | 25.61 | 0.269 |
| C | 40.18 | $7.066 \times 10^{-3}$ |
| D | 40.70 | $6.037 \times 10^{-3}$ |
| E | 43.71 | $3.849 \times 10^{-3}$ |

$\chi^2$ values are for 26 degrees of freedom; see text for details of models.
A, CDM + HDM, $\Omega_0 = 1$, $\Omega_{CDM} = 0.89$, $\Omega_\nu = 0.1$, $\Omega_b = 0.01$, $h = \frac{1}{2}$.
B, CDM + HDM, $\Omega_0 = 1$, $\Omega_{CDM} = 0.69$, $\Omega_\nu = 0.3$, $\Omega_b = 0.01$, $h = \frac{1}{2}$
C, Adiabatic CDM, $\Omega_0 = \Omega_{CDM} = 1.0$, $h = \frac{1}{2}$
D, Isocurvature CDM, $\Omega_0 = \Omega_{CDM} = 1.0$, $h = \frac{1}{2}$
E, Texture + CDM, $\Omega_0 = \Omega_{CDM} = 1.0$, $h = \frac{1}{2}$

The most satisfactory fit to the data is for the hybrid model with CDM and three neutrino species, two massless and one massive (HDM). We have not considered the case of three equally massive neutrinos because of the strong limits on the electron neutrino mass. Figure 1*c* shows the fit for two cases, with $\Omega_\nu = 0.3$ (solid curve) and 0.1 (broken curve). In both cases $\Omega_b = 0.01$ and $h = \frac{1}{2}$, with $\Omega_{CDM} = 0.69$ and 0.89, respectively. The $\Omega_\nu = 0.3$ model gives an excellent fit to all the data, whereas the $\Omega_\nu = 0.1$ case is marginally ruled out at the 99% level. This improved fit over the pure CDM model is due to the additional ingredient of damping on small-scales by neutrino free-streaming. The neutrino density parameter provides us with an estimate of the neutrino mass[38]

$$m_\nu = 0.1(\Omega_\nu h^2)\ \mathrm{keV} = 7.5\ \mathrm{eV} \qquad (10)$$

with a $1\sigma$ uncertainty of $\pm 2$ eV, well below the observational limits of 250 keV for the $\mu$-neutrino and 35 MeV for the $\tau$-neutrino. This model also gives an excellent fit to the shape of the APM angular correlation function, $w(\theta)$ (ref. 23). Although the neutrinos provide 30% of the mass in this model, they are responsible for the fit on scales $> 30h^{-1}$ Mpc. It would be interesting to see whether other mechanisms, besides CDM, are capable of reconciling HDM with observations. Provided that there has been no reionization, measurement of microwave background fluctuations on small angular scales ($\leq 1'$) would help to characterize nonlinear processes responsible for galaxy formation and small-scale clustering.

After submitting this paper for publication we received a copy of the paper by M. Davis *et al.* (preceding paper), which argues that a mixed hot and cold dark matter cosmology gives an excellent fit to galaxy clustering data. □

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# Circumstellar disks deduced from sub-arcsecond polarization observations of two young stars

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**HERBIG Ae/Be objects are young stars of intermediate mass (3–5 solar masses) surrounded by reflection nebulae composed of dust and gas remaining from the star-forming cloud[1–3]. Scattering by dust particles strongly polarizes light from the circumstellar nebula. Using imaging polarimetry in excellent conditions of atmospheric seeing (0.4 arcsec) from the La Palma observatory, we have resolved an optically thick disk around the Herbig Ae/Be object V376 Cassiopeiae. Its effective radius increases from 0.8 arcsec in the near infrared (~1 μm wavelength) to 1.2 arcsec at optical wavelengths (~550 nm) because of the higher opacity of the disk material at shorter wavelengths. The corresponding linear disk radius is 500–750 AU, about an order of magnitude larger than our Solar System. The polarization behaviour within a 0.5-arcsec diameter circle around the companion star V633 Cas suggests the presence of a more compact (<0.2 arcsec) unresolved disk, possibly a protoplanetary disk, around this star. The presence of circumstellar disks around these two pre-main-sequence stars in the same star-forming cloud suggests that such disks may be common features of early stellar evolution.**

Our imaging CCD polarimetry of the double Herbig emission source V376 Cas and V633 Cas was carried out on the night 1991 September 12–13 at the Nordic 2.56-m Optical Telescope (NOT) on La Palma[4]. The two sources are 37 arcsec apart on the sky and surrounded by a dense circumstellar cloud, producing an estimated extinction $A_V$ of about 5 and 7 mag for the central objects[5], which have apparent visual magnitudes of about 16 and 14, respectively. The complex is part of a larger dark cloud with a total estimated mass of 400–800 solar masses ($M_\odot$) and a diameter of ~2 pc (1 pc = 206,265 AU) at a distance of ~600 pc (refs 5, 6). Because of the high absorption produced by the circumstellar dust, a considerable part of the flux comes from the thermal infrared dust emission in the wavelength range 1–500 μm (refs 7–9). There is also a prominent molecular (CO) bipolar outflow[10–13] associated with V633 Cas (LkH$\alpha$ 198). According to estimates from infrared studies[9], V633 Cas is the more luminous of the sources, with $L \approx 250\ L_\odot$ (solar luminosity), whereas for V376 Cas, $L \approx 150\ L_\odot$.

The seeing conditions were excellent during our observations: the values measured from single exposures ranged from 0.4 to 0.6 arcsec. Figure 1*a* gives an intensity map of V633 Cas and the associated nebula. The broader profile of the central source (0.8 arcsec full width at half maximum) indicates that the object is not seen as a point-like star. The optically thick circumstellar matter absorbs most of the starlight and we see the surrounding diffuse envelope in multiply scattered light. The polarization map in Fig. 1*b* shows a pattern of centrosymmetrically oriented polarization directions around the source. This is consistent with single scattering of photons by dust particles in the optically thin outer parts of the envelope. The degree of polarization increases towards the southeast with the decreasing optical thickness of the cloud.

The companion star V376 Cas (Fig. 2*a*, enlarged) shows a central peak with two jet-like extensions, roughly in opposite directions. The 'jets' may in fact be two cones of light which escape from the central source in directions roughly perpendicular to an optically thick equatorial disk obscuring most of

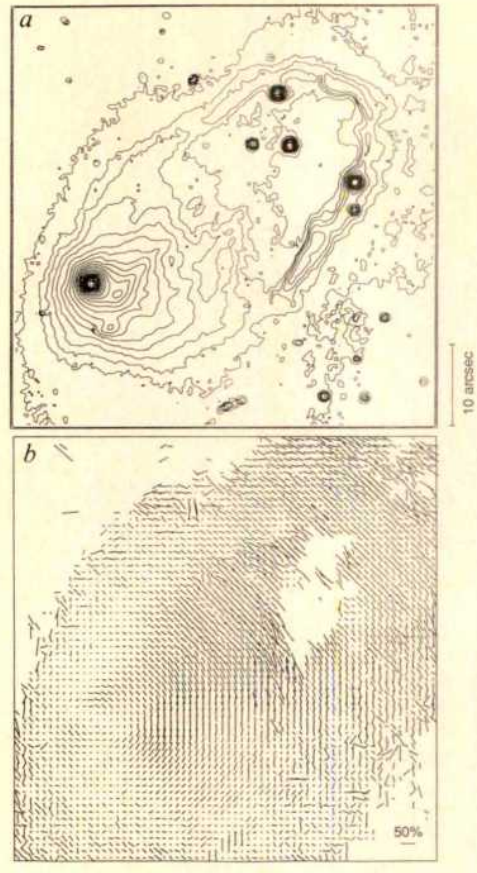


FIG. 1 *a*, I-band (900 nm) image of V633 Cas, contoured logarithmically to accommodate the large dynamic range between the faint nebulosity and the illuminating star. The intensity levels of the contours correspond to 44, 88, 125, 177, 250, 354, 500, 1,000, 2,000 and so on to 128,000 ADU (1 ADU ≈ 8.5 electrons is the readout unit of the CCD camera). The seeing value in this summed image of eight exposures is 0.6 arcsec (full width at half maximum). V633 Cas shows a broader central peak, indicating that this source, heavily obscured by the dust cloud, is not seen as a point-like star. *b*, Polarization map of the same area as *a*. Pixels have been averaged in blocks of 4 × 4 (0.8 × 0.8 arcsec) independently for each polarization direction given. The centrosymmetric pattern of the polarization vectors around V633 Cas can be explained by scattering of light by the dust particles in the circumstellar cloud. High polarization degrees (up to 50%) are seen in the optically thin outer parts, where single scattering dominates. South is up and east right in all figures.

the direct light[14–16]. The polarization map in Fig. 2*b* supports this interpretation, as the extensions are strongly polarized in the direction perpendicular to the line to the central star. This shows that the light we observe from the cones originates from the central object and is scattered towards us by the dust particles in the circumstellar medium. In the plane of the disk the polarization is smaller, because of multiple scattering.

There is a well defined area in the plane of the disk in Fig. 2*b*, where the polarization goes to zero. The distance of the 'null point', best seen in the southwest direction, is ~0.8 arcsec from the central object. As shown by Monte Carlo calculations (F. Menard, preprint from Département de Physique, Université de Montréal) the null polarization points occur at locations on the disk where the optical thickness changes from $\tau > 1$ (optically thick inner parts) to $\tau < 1$ (optically thin outer parts). The observed angular extent in the infrared I band (900 nm, Fig. 2*b*) corresponds to a radius of the dense parts of the disk of ~500 AU at a distance of 600 pc. In our V band (550 nm) polarization map, the area of aligned polarization directions in the centre is broader than in the I band, and the null points occur at a distance of ~1.2 arcsec, indicating that the optically thick part of the disk has a greater diameter at shorter wavelengths.

Figure 3 gives surface plots of the intensity distribution of the images of V376 Cas in the V and I bands, and of a field star for comparison. The effective diameter of the envelope is larger at 550 nm (V band) than at 900 nm (I band), consistent with the polarization data. Because the opacity of the circumstellar matter is higher at shorter wavelengths, the central object is not clearly visible in the V-band image, and the scattered light dominates. In the I band the central source shines through the dust (Figs 3 and 2*a*).

The larger optical thickness at shorter wavelengths could be explained by the dust particle population having a considerable component of small particles (<1 μm). In comparison, the optically thin circumstellar disk around $\beta$ Pictoris[17] has an intensity spectrum in the wavelength range 400–900 nm very close to that emitted by the central star[18], consistent with scattering particles much larger than 1 μm across. As $\beta$ Pic has already reached the main sequence and is thus older than V376 Cas and V633 Cas, there has been enough time for the small particles to be depleted, by radiation pressure (Poynting–Robertson effect), coagulation of small grains to form larger grains, or accretion onto larger grains.

An I-band polarization map of a 7 × 7-arcsec area centred on V633 Cas is shown in Fig. 4. The centrosymmetrically oriented polarization pattern dominates, except within the innermost 0.5-arcsec circle, where the polarization lines are parallel. This suggests the presence of a compact unresolved disk. Natta *et al.*[9] have included a disk component in their models for V633 Cas to explain the spectral energy distribution in the near-infrared wavelength range from ~1.6 to 10 μm.

Evidence of an unresolved polarized central source has also been found in infrared speckle observations at 1.6 μm (ref. 16). We have now detected this by direct imaging polarimetry at optical wavelengths with a ground-based telescope of high imaging quality. The polarization at 550 nm is larger (~12% at

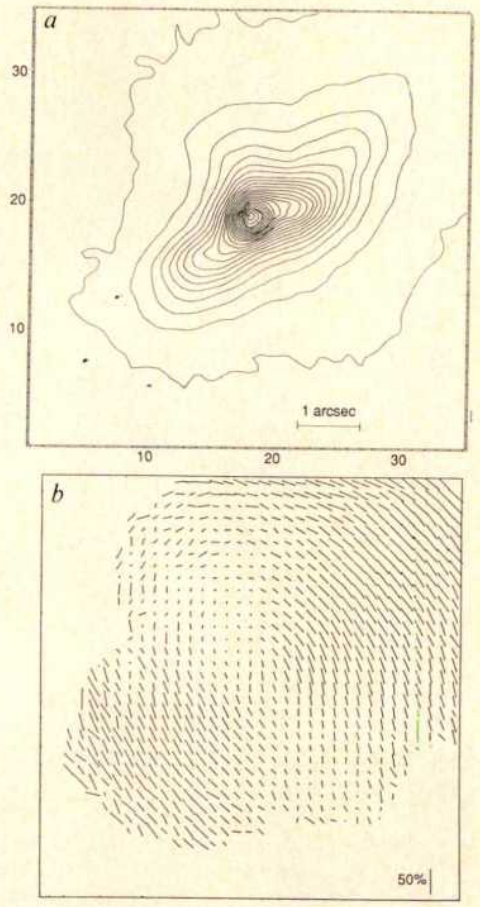


FIG. 2 *a*, Central portion (7 × 7 arcsec) of V376 Cas in the I-band (single 300-s exposure) linearly contoured from 50 to 2,450 ADU with 100 ADU interval to the individual pixels. A strong single peak with two jet-like extensions is seen. The seeing measured from the surrounding field stars in the same exposure is 0.50 arcsec (full width half maximum). *b*, Polarization map of the same area as *a*. Independent polarization directions have been calculated from each pixel (0.2 × 0.2 arcsec). The jet-like extensions are strongly polarized in a direction orthogonal to the line to the central source. In the plane perpendicular to the jets the polarization is smaller. This could be explained by multiple scattering in an optically thick disk around the recently formed star.

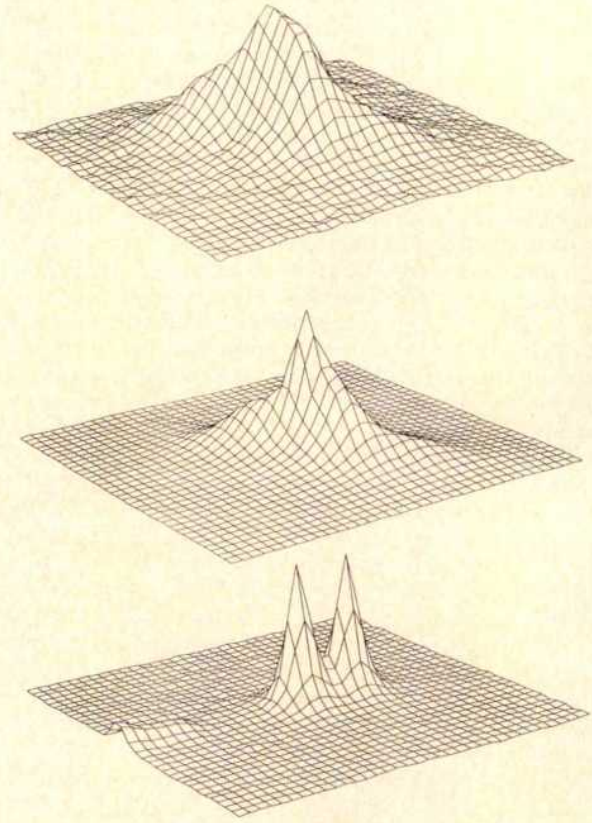

FIG. 3 Intensity plots of V376 Cas images (7 × 7 arcsec) at 550 nm (top) and 900 nm (middle), and an image (900 nm) of a field star, artificially doubled and shifted by 1 arcsec to produce a binary image (bottom). Note the greater opacity and extent of the circumstellar envelope at shorter wavelengths.

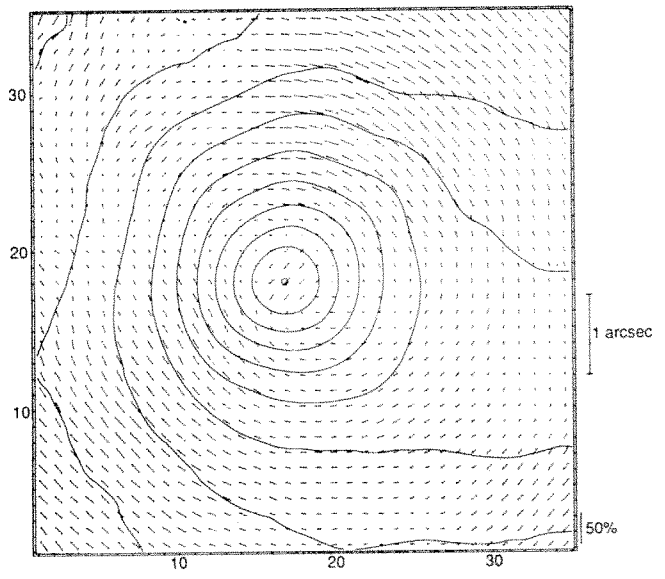


FIG. 4 Polarization map of an area of 7×7 arcsec centred on V633 Cas. Note the different polarization behaviour within the innermost 0.5-arcsec circle (aligned polarization directions), compared with the surrounding centrosymmetric pattern. This can be interpreted as being due to a compact, unresolved (radius < 0.2 arcsec) circumstellar disk around the central source. Contours give intensity levels decreasing by a factor of 2 from the peak.

138°, calculated from the flux within 0.25 arcsec from the peak) than at 1.6 μm (~6% at 154°). High degrees of polarization will result if the central source is obscured by the dense disk and the observed flux is dominantly scattered light from the flattened circumstellar envelope. The highly flattened disk required suggests that the material is associated with planet formation, rather than being expelled from the star.

The direction of the polarization integrated over an (unresolved) disk is sensitive to the density distribution of the scattering particles around the source, both in the disk plane and away from it. For moderate equatorial optical thicknesses the net polarization is axial (perpendicular to the projected disk plane), but it turns parallel to the disk plane for large optical depths, because of multiple scattering. From the present data on V633 Cas we cannot definitely distinguish between these two possibilities. Therefore, a 90° ambiguity remains for the orientation of the unresolved disk around V633 Cas. If the integrated disk polarization is axial, the projected orientations of the disk planes would be similar for V376 Cas and V633 Cas, having possible implications for the dynamical processes in the contracting protostellar cloud. □

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# Large anisotropic thermal conductivity in synthetic diamond films

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**As high-power electronic devices are packed to progressively higher densities, synthetic diamond films are being considered as heat spreaders for the prevention of thermal damage (see ref. 1 for example). Although diamond single crystals are known to have the highest thermal conductivity for any material at room temperature (22 W $cm^{-1}$ $K^{-1}$ for diamond with natural isotopic abundance, compared with 4 W $cm^{-1}$ $K^{-1}$ for copper), the dependence of conductivity on the microstructure of polycrystalline diamond films is not understood. Using a newly developed laser technique[2], we have measured thermal conductivity in the experimentally difficult direction perpendicular to the plane of the diamond film. Taken together with earlier in-plane measurements[3], this gives a complete description of the local thermal conductivity, showing a significant gradient and anisotropy correlated with the inhomogeneous grain structure. Despite phonon scattering at lattice defects and grain boundaries, we find that the local conductivity near the top growth surface of a synthetic diamond film is, surprisingly, at least as high as that of gem-quality diamond single crystals.**

Because most measurement techniques yield a conductivity that is averaged over the thickness $Z$ of the sample, we have extracted the local conductivity $\kappa_{\perp}^{\text{local}}$ for heat flow perpendicular to the film by measuring a series of samples of different $Z$. The gradient that we find can be compared with previous measurements[3] of the parallel conductivity $\kappa_{\parallel}^{\text{local}}$ on similar samples to reach a complete description of the local conductivity and its dependence on microstructure. This is interesting from a fundamental point of view as well as for thermal management of high-density, high-local-power electronic devices to prevent, for example, damage from self-heating. A diode laser, for instance, with a line of heat a few micrometres wide can be cooled effectively by mounting it against a diamond heat spreader.

The diamond films were nucleated and grown on commercially available single-crystal silicon substrates by microwave-enhanced plasma chemical vapour deposition (CVD) at 2.45 GHz. Depositions were done with a proprietary gas mix of hydrogen, hydrocarbons and an oxygen-bearing gas under conditions typical of diamond deposition using microwave CVD (pressures of ~100 Torr and temperatures between 800 and 900 °C). Except for differences in growth times and therefore the thickness $Z$, the deposition conditions were held constant from sample to sample. The aim was to produce a set of samples with a common microstructural profile (grain size, shape and orientation as a function of distance $Z$ above the substrate). The alternative approach of alternately measuring and thinning a single sample risks damaging the remaining material (but see below). The substrate was removed chemically, and the resulting films were optically transparent. Four samples, 0.5×1 $cm^2$ in area, with average thicknesses of 28.4, 69.1, 185 and 408 μm were evaluated. Figure 1 shows a fracture surface of the thickest sample (left) as well as top views of all four samples (right). The columnar nature of the microstructure and the growth of the grain size with $Z$ is clear. All films have a strong texture with ⟨110⟩ perpendicular to the substrate, and Raman scattering shows a strong diamond peak at 1,332 $cm^{-1}$ with little or no amorphous or graphitic component near 1,550 $cm^{-1}$. The mass density $\rho$ of each film, measured by means of Archimedes' principle, is within 1% of the bulk value, 3.51g $cm^{-3}$. The heat capacity $C_P$ per unit mass of similar films, measured (J.E.G.,

manuscript in preparation) in the temperature range 25–125 °C with an uncertainty of 2%, agrees with the bulk value[4]. Infrared absorption measurements (J.A. Mucha, manuscript in preparation) on the present films indicate a hydrogen concentration of ~0.1 atomic % which is the same for all samples within a factor of two. Electron paramagnetic resonance (EPR) measurements[5] on thin (25 μm) samples grown under similar conditions indicate that any nitrogen in the films occurs as point defects at a concentration of ~1 p.p.m.

To measure the thermal diffusivity $D_\perp$ through a thin film of highly conductive material, we have developed a high-speed laser flash technique[2] which is five orders of magnitude faster than a method originally developed for thick samples of ceramics[6] or bulk diamonds[7]. The transit time for a diffusive thermal pulse to pass through our thinnest sample is only ~200 ns. We therefore use an 8-ns pulse of 1.064-μm radiation from a Nd:YAG laser, absorbed in a 3,000 Å sputtered layer of titanium. A similar titanium layer on the top face allows fast thermometry with an infrared detector (cooled HgCdTe, 100-MHz bandwidth). The heating pulse is spread over a diameter of at least 3 mm, so that the arrival of the thermal step at the top face is well described by the solution[6] of the one-dimensional heat-flow equation for a sample of thickness $Z$

$$\Delta T(t) = A\left[1 + 2\sum_{n=1}^{\infty}(-1)^n \exp\left(-\frac{n^2\pi^2 D_\perp t}{Z^2}\right)\right] \qquad (1)$$

where $T$ is temperature and $A$ is the maximum temperature rise (the long-time limit of $\Delta T(t)$), determined by the amount of energy absorbed from the pulse per unit area and by the heat capacity of the sample. With signal averaging, a temperature resolution of 0.05 °C is achieved. A nonlinear least-squares fit of equation (1) to the data yields values for $D_\perp$ and $A$, and $\kappa_\perp$ is calculated from $\kappa_\perp = \rho D_\perp C_P$. This laser pulse technique has been tested for materials with a wide range of thermal conductivity: copper ($\kappa_{observed} = 4.0 \pm 0.1$ W cm$^{-1}$ K$^{-1}$) and synthetic single-crystal diamond isotopically enriched in $^{12}$C ($\kappa_{observed} = 33 \pm 1$ W cm$^{-1}$ K$^{-1}$, to be compared with 33 W cm$^{-1}$ K$^{-1}$ observed[8] by a different technique in similar material. A similar higher conductivity is expected for enriched CVD diamond, but this is very expensive to make). Anisotropy in the thermal conductivity was also found[2] in one film of CVD diamond using the present technique.

The conductivity data for $T = 25$ °C are shown in Fig. 2 for all four CVD diamond films. The fact that the observed conductivity $\kappa_\perp^{obs}$ depends on thickness shows immediately that the local conductivity depends on position $z$. It is possible to extract a local conductivity $\kappa_\perp^{local}(z)$ by comparing films of successive thickness. Because the heat pulse encounters successive layers in series, it is appropriate to define a resistance per square $R = Z/\kappa_\perp$ for a sample of thickness $Z$. For two samples of thicknesses $Z_i$ and $Z_j$ ($Z_j > Z_i$), the extra resistance per square of sample $j$ compared with sample $i$ is $Z_j/\kappa_{\perp j} - Z_i/\kappa_{\perp i}$, which can be defined as the average local resistance per square $R^{local}$ at height $\bar{z} = (Z_i + Z_j)/2$. The local conductivity at $\bar{z}$ is then $\kappa^{local} = (Z_j - Z_i)/(Z_j/\kappa_{\perp j} - Z_i/\kappa_{\perp i})$. Figure 2 shows the local conductivity calculated in this way. It rises more rapidly than the observed $\kappa_\perp$ (necessarily averaged over the thickness) and attains a value of 23.5 W cm$^{-1}$ K$^{-1}$. This can be compared with the value of 22 W cm$^{-1}$ K$^{-1}$ at 25 °C for type IIa single-crystal diamond, obtained as an average of several measurements[8–11]. The uncertainty for each local value is estimated at 5–7%, so that $\kappa^{local}$ near the top of the thickest sample is equal to or slightly greater than the conductivity of the best gem-quality diamond reported so far. After the measurements were completed, the thickest sample was carefully polished mechanically to remove 100 μm from the substrate side. On remeasuring, we found that $\kappa_\perp^{obs}$ had increased from 21 to 25 W cm$^{-1}$ K$^{-1}$ consistent with an average of $\kappa_\perp^{local}$ between $z = 100$ μm and $z = 400$ μm. The present results for $\kappa_\perp^{local}$ are compared in Fig. 3*a* with $\kappa_\parallel^{local}$, deduced recently[3] from measurements of $\kappa$ with heat flowing in the plane of the film using the same or similar samples. $\kappa_\parallel^{local}$ rises with $z$ much less rapidly than does $\kappa_\perp^{local}$, indicating a large anisotropy in $\kappa^{local}$ for $10 \leqslant z \leqslant 200$ μm. The conductivity near the bottom is consistent with $\kappa_\parallel = 2$–$6$ W cm$^{-1}$ K$^{-1}$ observed[12] for films with thickness in the range 3–13 μm.

To understand these data, we recall[13] that for insulating crystals heat is carried by phonons whose mean free path is limited by various scattering mechanisms, including scattering by (1) other high-energy phonons (intrinsic, *umklapp* scattering),

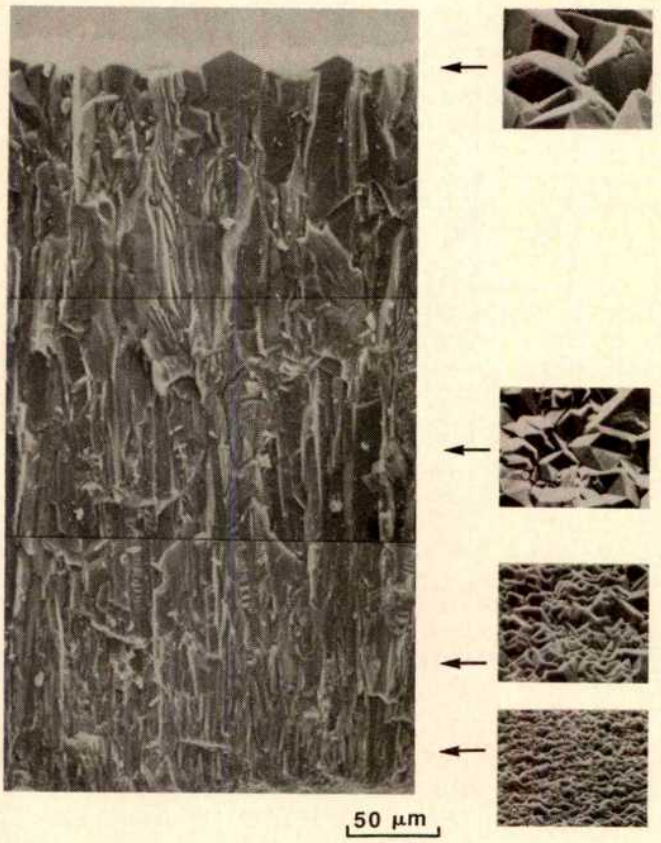


FIG. 1 Left, scanning electron micrograph of a fracture surface of the 408-μm-thick sample. Right, top views of each of the four samples, showing the dependence of grain size on the height above the substrate.

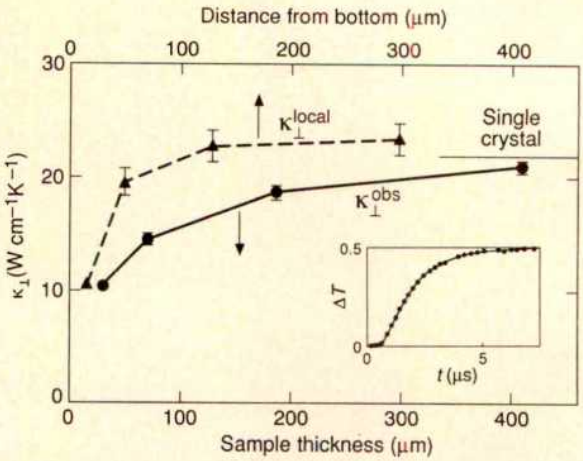


FIG. 2 Average (observed) and local (deduced) conductivity for heat flow perpendicular to the plane of the film plotted as a function of thickness or distance from the bottom of the sample. Inset, typical raw data for $\kappa_\perp^{obs}$.

(2) impurities (Rayleigh scattering from point defects), (3) lattice defects such as dislocations or other extended defects, and (4) boundaries. The standard relaxation-rate approach within the Debye model has already been applied[14] to data for $\kappa_\parallel$ of our thickest sample. In the temperature range 4–400 K, all of the above scattering mechanisms were needed to fit the data. Similar results for $\kappa_\parallel$ in other high-quality CVD diamond have been obtained[15]. We have measured $\kappa_\parallel$ for all of the present samples down to liquid helium temperatures and extracted $\kappa_\parallel^{local}(z, T)$, which is then analysed by the standard model to obtain scattering strengths as a function of height $z$. The results (J.E.G., manuscript in preparation) show that at room temperature the thermal conductivity can be modelled by scattering from point defects, extended defects (diameter ~12 Å), dislocations and grain boundaries. Each of the first three mechanisms contributes a thermal resistance roughly twice the resistance attributable to boundary scattering, and all four become less important with increasing $z$. The latter finding is consistent with the observed gradient in conductivity. The observed anisotropy, however, is not so readily explained, as one would expect the scattering from point defects or roughly spherical extended objects to be isotropic. Consideration of the microstructure (Fig. 3*b*) suggests that the anisotropy can be explained by assuming a concentration of defects at or near grain boundaries for $z \lesssim 250$ μm. Transmission electron microscopy[16] shows a tendency for lattice imperfections in CVD diamond to be concentrated at grain boundaries. Such clustering of defects would produce a higher probability that a phonon incident on a grain boundary would be reflected rather than scattered isotropically. A decreased transmission through grain boundaries would impede parallel thermal transport more than perpendicular, providing a natural explanation for the anisotropy.

A year ago it was not expected that the thermal conductivity of CVD diamond would ever rival that of good single crystals. The present results show that the defect concentration of state-of-the-art material is already low enough to obtin a conductivity equal to or exceeding that of the best gem-quality bulk diamond. □

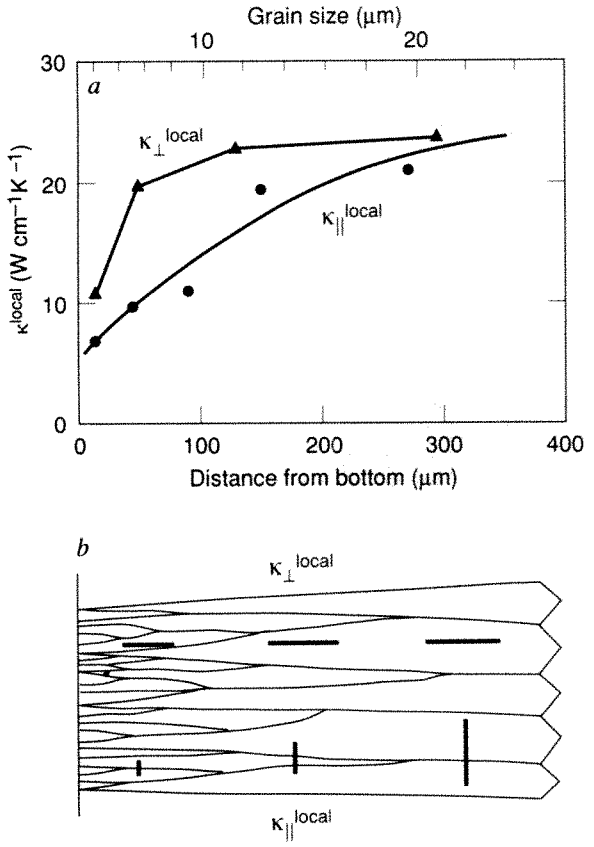


FIG. 3 *a*, Local conductivity for heat flow perpendicular to the film, $\kappa_\perp^{local}$ (from Fig. 2) compared with that for heat flow parallel to the film $\kappa_\parallel^{local}$ (ref. 3). The curved line through the $\kappa_\parallel^{local}$ points was derived from a polynomial fit to the raw data. The nonlinear top scale gives the grain size as a function of distance from the bottom of the samples (bottom scale), obtained from the micrographs of Fig. 1. *b*, Schematic view of the typical cone-shaped grain structure of CVD diamond films. The lengths of the bars are proportional to local conductivity for heat flow perpendicular ($\kappa_\perp^{local}$) and parallel ($\kappa_\parallel^{local}$) to the plane of the film.

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# A decrease in the growth rates of atmospheric halon concentrations

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**HALONS H-1301 ($CBrF_3$) and H-1211 ($CBrClF_2$) have been introduced into the atmosphere, mainly through use in fire extinguishers, for almost three decades. Although each is now present in the troposphere at a concentration of only 2 parts per $10^{12}$, these gases have long atmospheric lifetimes (65–77 yr for H-1301 and 11–16 yr for H-1211)[1,2] and carry significant amounts of bromine to the stratosphere[2,3], where it can destroy ozone catalytically[4,5]. For this reason, the halons have high ozone depletion potentials[6]. The manufacture of both gases is to be discontinued globally by the year 2000, according to the Montreal Protocol, and perhaps sooner, as a result of unilateral action by users, manufacturers and producing countries[7,8]. Here we present a six-year record of tropospheric halon mixing ratios which shows that the growth rates of H-1301 and H-1211 have already begun to decrease substantially. This recent decrease in growth rates is consistent with industry emission estimates (although these have greater uncertainties), and supports current appraisals of atmospheric lifetimes. Our results suggest that, even though these halons are relatively long-lived species, their atmospheric mixing ratios may stabilize or begin to decrease within the next few years.**

Measurements of both halons were made from flasks collected at NOAA's climate monitoring stations and cooperative flask

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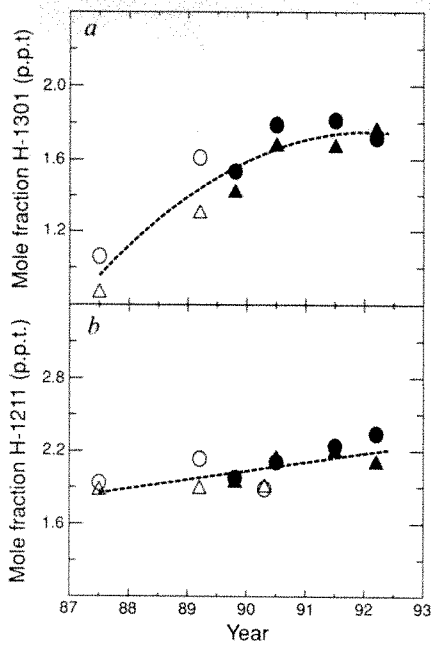


FIG. 1 Dry atmospheric mixing ratios of (*a*) H-1301 and (*b*) H-1211 from 1987 to 1992. Circles denote the Northern Hemisphere and triangles represent the Southern Hemisphere. Open symbols are latitudinally weighted means of data from cruises in the remote Pacific Ocean; filled symbols are latitudinally weighted, calendar-year averages of monthly measurements from fixed sites. (Measurements of H-1301 were not obtained for the 1990 cruise.) The standard error of each mean is represented by the size of the symbols. With $t=0$ in mid-1989 (at 1989.5), the fitted curve for H-1301 is $X_{H\text{-}1301}=1.50\ (\pm0.05)+0.196\ (\pm0.024)t-0.0383\ (\pm0.0141)t^2$ and has a residual standard deviation on $X_{H\text{-}1301}$ of 0.11 p.p.t. The corresponding fitted line for H-1211 is $X_{H\text{-}1211}=2.00\ (\pm0.03)+0.0725\ (\pm0.021)t$ with a residual standard deviation on $X_{H\text{-}1211}$ of 0.11 p.p.t.

sampling sites, and on research cruises in the Pacific Ocean. Paired samples were collected every 1–2 months in electropolished, stainless-steel flasks from mid-1989 to early 1992 at four of the five CMDL flask network sites: Point Barrow, Alaska (71.3° N, 156.6° W); Niwot Ridge, Colorado (40.1° N, 105.5° W); Mauna Loa, Hawaii (19.5° N, 155.6° W); and American Samoa (14.3° S, 170.6° W). The fifth station, located at the South Pole (90° S), was sampled only during the summers of two years, so the record from that station is not contiguous. In 1991 sample collection for halons was begun at Alert, Canada (82.5° N, 62.3° W) and Cape Grim, Tasmania (40.7° S, 144.8° E). A total of 60 flasks was filled on research cruises in May–July 1987 (45° N to 30° S), February–April 1989 (50° N to 60° S), and February–April 1990 (20° N to 15° S) in the remote Pacific Ocean.

Subsamples (~100 ml) from the flasks were cryo-trapped on alumina-lined fused-silica tubing and analysed for both halons by temperature-programmed, electron-capture gas chromatography (ECGC)[9]. In 1992, 250-ml subsamples were also cryo-trapped on uncoated fused-silica tubing and analysed for H-1211 by gas chromatography mass spectrometry (GCMS). Measurements between the two systems agreed within ±0.1 parts per $10^{12}$ (p.p.t.), which is within 1 s.d. All mixing ratios are traceable to gravimetrically prepared standards, made up in our laboratory. This procedure for preparing standards is similar to that used by the National Institute for Standards and Technology[10] and typically is accurate to within ±1%. The coefficient of variation for repeat measurements of these samples was about 5%.

The mean difference in H-1301 mixing ratio between samples collected within the same latitudinal bands on the first two cruises suggests a growth rate of $0.29\pm0.04$ p.p.t. $yr^{-1}$ for 1987–1989 (Fig. 1). This high growth rate is also supported by the mean interhemispheric difference of 0.28 p.p.t. in the cruise samples. Because the atmospheric lifetime for H-1301 is long compared with the interhemispheric exchange time of ~1 yr (refs 11, 12) and because it is likely that most of the H-1301 is emitted in the Northern Hemisphere, the latitudinally weighted, annually averaged, mean interhemispheric difference should roughly equal the annual growth rate (see Table 1). Strictly calculated, this would represent a growth rate of ~0.25 p.p.t. $yr^{-1}$ during this interval.

Data from the CMDL flask network show a significantly lower rate of growth for H-1301 from 1989 to 1992 (Fig. 1*a*; Table 1). The rate of increase varied among individual stations from 0.11 to 0.19 p.p.t. $yr^{-1}$, with a mean of $0.14\pm0.03$ p.p.t. $yr^{-1}$. The mean interhemispheric difference during this time was 0.18 p.p.t., which represents an atmospheric growth rate of 0.15 p.p.t. $yr^{-1}$ according to the model in Table 1, and is consistent with the observed trends.

The growth rate of atmospheric H-1211 was relatively constant at $0.07\pm0.02$ p.p.t. $yr^{-1}$ for 1987–1992 (Fig. 1*b*). Although the mean interhemispheric difference for H-1211 of 0.11 p.p.t. supports an atmospheric growth rate much closer to zero, H-1211 is not as well suited to this calculation as is H-1301; the lifetime of H-1211 is much shorter, but, more importantly, its emission pattern may be more variable, as it is used mainly in portable fire extinguishers. The mean growth rate of 0.07 p.p.t. $yr^{-1}$, however, is much less than those reported by other investigators for earlier periods (Table 1). It is clear from these data that the atmospheric growth rates of both halons have decreased markedly in recent years, both in absolute and relative terms.

Production of both halons began declining after 1988, but it is less certain exactly when, and to what extent, releases started to decrease. Using emission data given by McCulloch[13], we calculated annual growth rates from 1960 to 1990 with a simple,

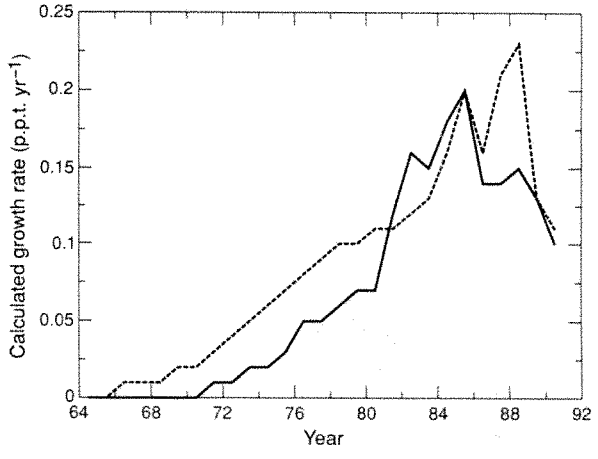


FIG. 2 Tropospheric growth rates for H-1301 (solid line) and H-1211 (dashed line), calculated from emission data given in ref. 13.

TABLE 1 Atmospheric growth rates of H-1301 and H-1211 in 1978–1992

| Year | Location | H-1301 (p.p.t. $yr^{-1}$) | H-1301 (% $yr^{-1}$) | H-1211 (p.p.t. $yr^{-1}$) | H-1211 (% $yr^{-1}$) |
|---|---|---|---|---|---|
| 81 | Pacific Ocean[25]* | 0.1 | 13 | — | — |
| 82–84 | Upper troposphere[14] | — | — | 0.12 | 20 |
| 79–85 | South Pole[26]† | — | — | 0.16 | 21 |
| 79–87 | Upper troposphere[15]†‡ | 0.05 | 4.5 | 0.16 | 12 |
| 78–87 | Oregon coast[27]† | 0.05–0.21 | 18 | 0.16 ± 0.01 | 7–15 |
| 87–89 | Pacific Ocean | 0.29 ± 0.04 | 16 | 0.07 ± 0.02 | 3 |
| 89–92 | CMDL observatories | 0.14 ± 0.03 | 8 | | |

* Growth rate estimated from interhemispheric difference is

$$\frac{dX_s}{dt} = \frac{\Delta X}{\tau_e} - \frac{X_s}{\tau}$$

where $X_s$ is the halon mixing ratio in the Southern Hemisphere (p.p.t.), $\Delta X$ is the latitudinally weighted interhemispheric difference in mixing ratio (p.p.t.), $\tau_e$ is the interhemispheric exchange time, taken to be 1 yr, and $\tau$ is the atmospheric lifetime of the halon. This equation, which requires that emissions in the Southern Hemisphere are insignificant, is derived from a simple, two-box, mass balance model[28]. $dX_s/dt$, the growth rate in the Southern Hemisphere, is a close approximation to the total atmospheric growth rate for the halons and is within the uncertainty of the measurements.

† Because data from the various studies were not standardized by intercalibration, the absolute growth rates have been adjusted to match our scale, where possible. Relative growth rates (% $yr^{-1}$), however, are not affected by differences in calibration scales.

‡ On the basis of only three data points; large relative uncertainty in mixing ratios.

one-box, finite-increment model, derived from the mass balance of atmospheric growth, emissions and destruction

$$\Delta X(t+1) = \frac{\Delta t}{\tau}\left[(\tau - 0.5)\frac{fE(t+1)}{M_a} - X(t)\right]$$

where $\Delta X(t+1)$ is the mean change in tropospheric mixing ratio for time increment $t+1$, $E(t+1)$ is the total annual emission, $M_a$ is the mass of the atmosphere, $X(t)$ is the average tropospheric mixing ratio at the end of a given year, $\tau$ is the mean lifetime in the atmosphere, $\Delta t$ is one year, and $f$ is the fraction of total atmospheric halon in the troposphere divided by the fraction of total atmospheric mass in the troposphere. The $(\tau - 0.5)$ term accounts for the fact that emissions occur throughout the year, rather than in a pulse at the beginning of the year. $f$ was calculated as 1.13 for H-1301 and 1.17 for H-1211 from data given in refs 14 and 15. The emission models predict mixing ratios well within the combined uncertainties for emission estimates and for our measurements, indicating good agreement among our data, industry assessments of production and emission rates[13], and published atmospheric lifetimes[1,2].

The recent downward trends in the growth rates of both halons are also supported by emission data, although there is occasional disagreement in the absolute annual rates (Fig. 2; Table 1). Growth rates calculated from atmospheric data are associated with some error, but the uncertainties in estimating annual emissions from production figures are much greater. This is particularly true for the late 1980s and early 1990s, when mandatory and voluntary reductions in use of the halons have markedly reduced their release to the atmosphere. McCulloch[13] simply estimated emissions as fractions of production over three intervals from 1960 through 1990. Today, considerable effort is being made to limit releases[16–18], and much of the halon already produced remains in extinguishers or has been stockpiled. Recent growth rates calculated from measurements of atmospheric mixing ratios are probably more reliable than estimates from release scenarios.

Halons in the troposphere contribute significant amounts of bromine to the stratosphere. Together, H-1211 and H-1301 amount to just over 4 p.p.t. in the troposphere. H-2402 ($C_2Br_2F_4$), a halon produced primarily in Japan and the former Soviet Union, is believed to be present at 0.5–1.0 p.p.t., but little information is available for this compound. The only other main source of bromine to the stratosphere is tropospheric $CH_3Br$, a gas with both natural and anthropogenic sources and an atmospheric lifetime of ~1.8 yr (ref. 19); other bromine-containing compounds, such as $CHBr_3$, are too short-lived to deliver much bromine to the stratosphere[20]. Reported tropospheric mixing ratios of $CH_3Br$ range from two to hundreds of parts per $10^{12}$, depending mainly on proximity to anthropogenic sources[21]. Cicerone *et al.*[22], however, examined a 3-yr record of $CH_3Br$ measurements from remote locations, finding a variation of only 20% in the mean monthly averages at each station. The range of means over the globe was 9.5–11.5 p.p.t. and no significant growth was observed at any site. Salawitch *et al.*[3] suggested that the most probable mixing ratio of total stratospheric bromine is 16 ± 4 p.p.t., so H-1301 and H-1211 must amount to 20–30% of the total bromine delivered to the lower stratosphere. Prather *et al.*[2] suggested that all halons contributed up to 40% of stratospheric bromine. The decline in both production and atmospheric growth rates of halons reduces the probability of runaway growth of atmospheric bromine[23,24], but it also is likely that halon emissions will continue, owing to leakage and periodic use, long after production has ceased. Nevertheless, atmospheric data show that growth rates of the halons are declining under present releases. If reductions in use continue as they have in recent years, the atmospheric mixing ratios of H-1211 and H-1301 could begin dropping as early as 1994 or 1995. □

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# The planform of compositional convection and chimney formation in a mushy layer

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COMPOSITIONAL **convection occurs when a liquid containing more than one chemical component undergoes fractional crystallization. Above a critical solidification rate, a solid–liquid mixed phase (or 'mush') develops which can exhibit spatial gradients of permeability and flow due to preferential dissolution and precipitation in the upwellings and downwellings respectively. A striking, but poorly understood, example is when upflow occurs in narrow, crystal-free, cylindrical channels or 'chimneys'. Such dynamic effects may occur in the Earth's core[1], crustal magma reservoirs[2], hydrothermal systems at mid-ocean ridges[3] and during the diagenesis of sedimentary rocks[4]. Using experiments designed to maximize the effects of dissolution and precipitation, we show that chimney formation can be related to a known planform of convection. Just above marginal stability, upwelling (and dissolution) occurs along the perimeters of hexagonal cells, with downwelling (and precipitation) at the centres, giving rise to a tessellated network of vertical, crystal-free channels. Subsequent focusing of the upflow at the nodes of the hexagons and recrystallization in the linear channels results in isolated chimneys at the nodal positions.**

Compositional convection is often seen when a multicomponent liquid is cooled from below at a horizontal boundary and an unstable density gradient results because the residual liquid produced by crystallization, although colder, is sufficiently depleted in the heavier component. For a binary system, one writes the liquid density ($\rho$) as

$$\rho = \rho_0\{1 - \alpha(T - T_0) + \beta(C - C_0)\} \quad (1)$$

where $T$ is the temperature, $C$ the concentration of the heavier component, $\rho_0$ the density at temperature $T_0$ and composition $C_0$, $\alpha$ the thermal expansion coefficient and $\beta$ its compositional analogue. A key aspect of crystallization is the formation, above a critical growth rate, of a 'mush' (Fig. 1*a*), which has irregular microscopic geometry. Equations have been proposed[5] which treat the mush as a porous region. Experiments with aqueous solutions have shown that the two phases approach local thermodynamic equilibrium[6,7].

When a mush forms, convective instability is possible both in the thin chemical boundary layer just above the mush and in the interstitial liquid (Fig. 1*a*). The former is easily observed[8], but the latter is hard to characterize. In ref. 7 the onset of convection was found to be associated with the development of weak porosity fluctuations in the mush and of a hummocky appearance of its previously flat upper surface. These observations indicated that instability occurs when the Rayleigh number of the porous medium reaches a critical value

$$\mathrm{Ra_p} = \frac{g\Delta\rho\Pi_0 h}{\kappa\mu} = \mathrm{Ra\ (crit)} \quad (2)$$

where $h$ is the mush thickness, $\Delta\rho$ is the density difference across the mush, $\kappa$ the thermal diffusivity, $\mu$ the viscosity and $\Pi_0$ a representative permeability. Assuming local equilibrium, the temperature and composition of the interstitial liquid are related by

$$T - T_s = \Gamma(C - C_s) \quad (3)$$

where $\Gamma$ is a constant for the $NH_4Cl$ solutions used. $T_s$ is the melting temperature of pure solid (composition $C_s$).

The key idea for understanding convection in the mush is

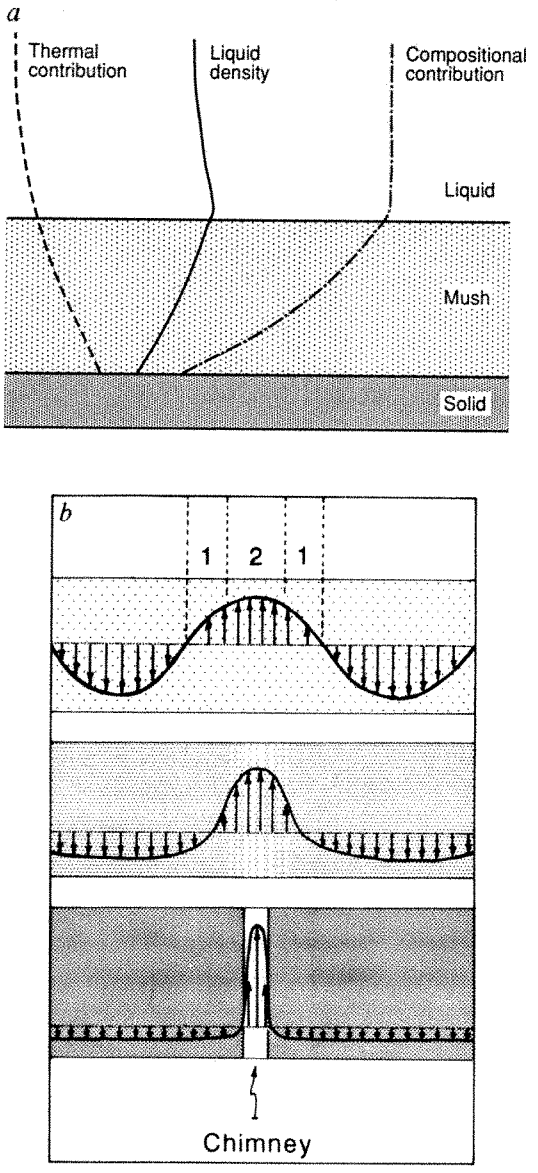



FIG. 1 *a*, Cooling from below leads to a positive temperature gradient and to a positive composition gradient because fractional crystallisation depletes the residual liquid in heavy component. The bulk density gradient (solid line) is the result of the thermal contribution (dashed line) and the compositional contribution (dot-dashed line). *b*, Schematic diagram of the mechanism suggested for the formation of chimneys. Just above marginal stability the porosity is the same in upwellings and downwellings. At the edges of upwellings (region 1), cooling dominates dissolution and porosity decreases. In the centres of upwellings where the vertical velocity is greatest (region 2), dissolution dominates and porosity increases. The horizontal gradients of permeability cause the flow progressively to focus.

that of porosity fluctuations. Chemical diffusion can be neglected[9]; in this limit the equation for composition becomes

$$\chi\frac{\partial}{\partial t}(C - C_s) = -\mathbf{U}\cdot\nabla(C - C_s) - (C - C_s)\frac{\partial\chi}{\partial t} \quad (4)$$

where $\mathbf{U}$ is the Darcy velocity and $\chi$ is the porosity, as we have assumed solid and liquid to have equal densities. A more general treatment might include a compaction term in equation (4). This effect did not occur in our experiments, described below, and although interesting, analysis of this effect is beyond the scope of the present paper. Using equation (3) and the fact that the vertical temperature gradient is much larger than the horizontal

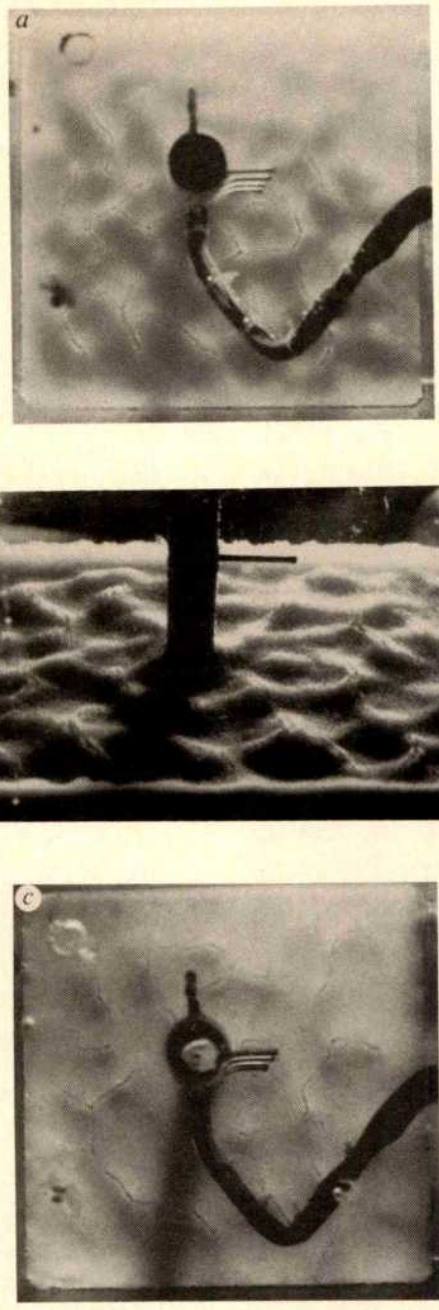


FIG. 2 *a*, View from above showing the cellular pattern which is visualized by porosity variations. Upwellings are the dark perimeters of the cells where crystals have been dissolved. The frame size is roughly 20 × 20 cm. *b*, View from the side showing the morphology associated with the cellular pattern. *c*, View from above later on showing that branches have disappeared to produce fewer larger cells.

gradients, equation (4) reduces to

$$\frac{\partial \chi}{\partial t} = -\frac{\chi}{(T-T_s)}\frac{\partial}{\partial t}(T-T_s) - \frac{W}{(T-T_s)}\frac{\partial}{\partial z}(T-T_s) \qquad (5)$$

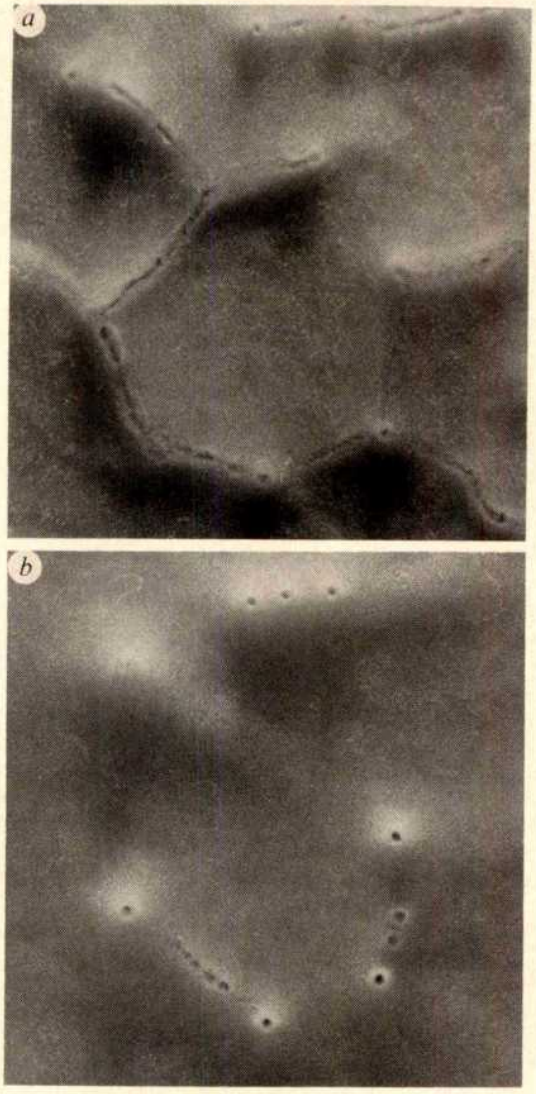
Before motion occurs, the second term on the right-hand side is zero and cooling causes porosity to decrease everywhere. Once convection begins, porosity fluctuations occur because the sign of the second term depends on the sign of $W$, the vertical velocity. In downwellings, both terms are negative and porosity always decreases. In upwellings, the terms have opposite signs. Near the edge of an upwelling, $W$ is small, the cooling term is larger and porosity tends to decrease, albeit less rapidly than in downwellings. At the centres of upwellings, $W$ is largest, and here advection can dominate cooling and porosity can increase (Fig. 1*b*).

Equation (5) says that dissolution and precipitation are the means by which the solute budget is closed. As fluid rises through the temperature gradient, it heats up, and downwelling fluid is cooled. The condition of local equilibrium requires the composition of the fluid to adjust, by dissolution in the upwellings and precipitation in the downwellings. Horizontal gradients of permeability develop, and we expect a feedback effect whereby the intensity of the flow increases in the higher-permeability upwellings and decreases in downwellings (Fig. 1*b*). We now present experiments that relate chimneys to the planform of porous medium convection. At instability, all planforms of regular polygons which tesselate the plane are possible, but nonlinear factors such as the temperature dependence of viscosity lead to a particular planform being favoured[10]. For example, hexagons have been observed experimentally when thermal convection occurs in nonreactive porous media[11,12].

We used 28 wt% aqueous solutions of ammonium chloride ($NH_4Cl$) whose liquidus temperature is 23.7 °C. Initial temperatures were ~24–27 °C, representing small degrees of superheat. We reduced the temperature of the base plate slowly (typically 1 °C per 5–10 min) until a specified final value (~5 °C) was reached. Crystals nucleated and a mush grew on the base of the tank. The boundary layer at the top of the mush become unstable, leading to convection in the overlying fluid, although the mush remained flat and homogeneous. Porosity fluctuations became visible in the upper surface, signalling the onset of convection in the porous medium, typically when the mush was 1.3–1.5 cm thick. At this moment, cooling rates in the mush (for example, 1 cm above the base plate) were 0.02–0.1 °C $min^{-1}$. Shortly afterwards, linear structures with various orientations appeared which became progressively more marked until finally a cellular planform developed (Fig. 2*a*). At the top of the mush, the edges of the cells were higher than the centres (Fig. 2*b*). The cellular pattern evolved as the mush thickened. Branches of the cell perimeters became choked with crystals and the associated morphology disappeared. The pattern changed mostly by loss of branches rather than by migration, resulting in a steady increase in cell size (Fig. 2*c*). The maximum width attained by the channels was 1–2 mm. At the nodes, dissolution structures were wider than along the sides (Fig. 3*a*). The dissolution structures eventually disappeared except for chimneys which remained where the nodes of the cells had been (Fig. 3*b*).

We characterized the planform geometry from photographs taken just after it became visible. Here we describe the example shown in Fig. 2*a* which is typical. First, we evaluated the number of branches intersecting one another at the nodal points. For ideal patterns, this gives 6 for triangles, 4 for squares and 3 for hexagons. In reality, there were some junctions with missing branches, either terminations or elbows. Excluding these from the count gave a value of 3.2, and including them gave a value of 2.8, both of which are close to that for hexagons. We then calculated the average length of the sides. The average cell area was 11.5–15.3 $cm^2$ (allowing for subjective judgement of a few imperfectly developed cells). For regular hexagons, this implies sides in the range 2.1 to 2.4 cm. Direct measurement gave an average length of 2.2 cm.

Comparison with theoretical predictions of the onset of convection requires caution because calculations have not been done for the exact thermal conditions of our experiments and hence there are differences in the reference vertical profile of permeability in the mush at marginal stability[13]. Permeability is a rapidly varying function at high porosity, exacerbating the problem of defining a scale value in order to calculate the local Rayleigh number. Using the same definitions as in ref. 7 we found a critical Rayleigh number of 80 ± 10. Values calculated for the special case of a mush growing at a constant rate at similar superheat[13] are lower by a factor of 3. Nield calculated



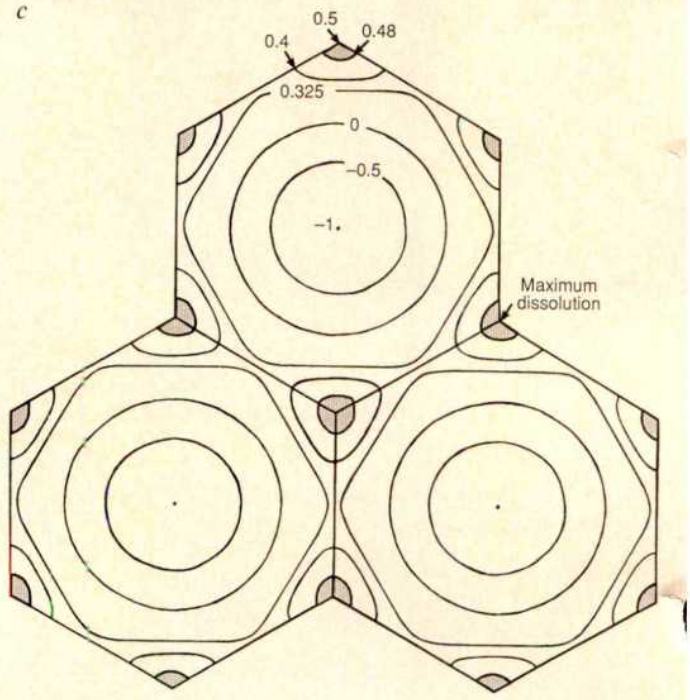




FIG. 3 *a*, Close up of a cell. Note the widening of the dissolution structures at the vertices. *b*, Some time later the branches have all been filled in and only chimneys remain at the original positions of the cell vertices. *c*, Contours of vertical velocity for a periodic hexagonal planform in a fluid undergoing thermal convection (after ref. 18). Velocities are normalized to a magnitude of 1 at the centre of the cell where downwelling occurs. The maximum upward vertical velocity, and hence maximum dissolution, occurs at the vertices. Comparison with (*a*) shows how the dissolution structures outline the velocity field.

the critical wavenumber for thermosolutal convection in a porous medium of constant permeability[14]. In the case of a mush with a vertical permeability gradient[13], the limit of Nield's value is rapidly approached, as the temperature difference decreases, implying a weaker permeability gradient. The expected dimensionless wavenumber in our experiments is in the range $\pi/2 < k < 2$. The length of a segment for the hexagonal planform is

$$\frac{\lambda}{3} = \frac{\lambda' h}{3} = \frac{2\pi}{3k} h \qquad (6)$$

where $\lambda$ is the wavelength. Measurements were made when the mush thickness was 1.8 cm. The predicted side length is therefore in the range 1.9–2.4 cm, in good agreement with the observations.

The vertical velocity ($W$) for a hexagonal planform given by[15]

$$W = f(z)\left\{2\cos\frac{2\pi\sqrt{3}}{\lambda}x\cos\frac{2\pi}{\lambda}y + \cos\frac{4\pi}{\lambda}y\right\} \qquad (7)$$

Figure 3*c* shows contours of $W$, and comparison with Fig. 3*a* shows how nicely the dissolution structures reflects the flow field as is implied by equation (5). We suggest that in previous experiments (for example refs 7, 16, 17), cooling was more rapid and dissolution structures were able to develop only at the nodes whereas here they develop along the sides and make the pattern visible. In nonreactive porous media[11,12], convective upwelling takes place at the centres of the cells, and downwelling at the perimeters. In our experiments, flow was always in the opposite sense, presumably because of the nonlinear effects of permeability variations. It has been suggested that convection produces chimneys by shearing of dendritic side branches and also that they nucleate on convective instabilities at the top of the mush and propagate downwards[16,17]. Our experiments indicate that, on the contrary, the key processes are dissolution and precipitation related to the velocity field, and that the formation of chimneys involved recrystallization along the edges of convection cells. In similar experiments with other compounds, chimneys have not been observed. The capacity of a given system to form chimneys depends on its reaching the critical Rayleigh number for convection and on the subsequent porosity evolution in the nonlinear regime, which depends on the cooling conditions. □

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# Parents suppress reproduction and stimulate dispersal in opposite-sex juvenile white-footed mice

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**JUVENILE dispersal is sex-biased in many mammals and birds: one sex often disperses more often or farther than the other. Two hypotheses are generally presented for sex-biased dispersal. The first holds that juvenile dispersal reduces reproductive and/or resource competition between parents and same-sexed offspring[1-5]. If so, presence of a parent on the natal home range should both promote dispersal of same-sex offspring and suppress reproduction of those that remain. The second is that juvenile dispersal reduces matings between parents and offspring[6-10], thus decreasing the likelihood of inbreeding depression[11-13]. If so, presence of a parent should favour dispersal and reproductive suppression of offspring of the opposite sex. Here I present evidence that juvenile dispersal in white-footed mice, *Peromyscus leucopus*, is due to inbreeding avoidance. When population density was high, experimental removal of one parent delayed dispersal of opposite-sexed offspring and only the presence of the parents of opposite sex suppressed juvenile reproduction.**

My studies of deermice, *P. maniculatus*, and white-footed mice at the Mountain Lake Biologial Station, Virginia (37° 10′ N, 80° 30′ W)[6,14-18], showed that juvenile dispersal is male-biased, although some females also disperse[6]. Spring-born juveniles usually become sexually mature and breed by the autumn of their first year, but reproductive suppression of unknown cause seemingly occurs at high population densities[16]. In April-October 1989-1991, I used standard mark-and-recapture live-trapping techniques[14,15] and 100 nest boxes to identify and monitor mothers, putative fathers and offspring on three 2.25-ha grids.

Density of *Peromyscus* during the breeding season averaged 30 mice per ha in 1989, 103 per ha in 1990 and 31 per ha in 1991. The two species are similar in behaviour and ecology in this area[14-18], but I present results only for white-footed mice. In 1989 and 1990, 85 mothers with 181 sons and 165 daughters were observed in nest boxes. Over this time, median residency for juveniles in natal home ranges increased from 1 to 6 weeks for males (Mann-Whitney $U = 820$, $P = 0.000$), and from 2 to 5 weeks for females ($U = 1{,}167$, $P = 0.109$). The proportion of juveniles remaining in natal home ranges long enough to become sexually mature (≥7 weeks) increased from 16 to 49% for sons ($\chi^2 = 11.45$, $P = 0.001$) and from 29 to 48% for daughters ($\chi^2 = 3.372$, $P = 0.066$; Table 1). This reduced dispersal rate coincided with an increase in space occupied by territorial resident adults from 50-70% in 1989 to 100% in 1990. Thus, as population density increased and no suitable habitat was available for colonization, juveniles remained in their natal sites, resulting in the number of mice reaching sexual maturity decreasing from 100 to 8% for sons (Fisher's exact test, $P = 0.000$) and 78 to 31% for daughters ($\chi^2 = 5.049$, $P = 0.025$; Table 1).

The probability of any individual becoming sexually mature in its birth year was dependent in part on its social environment. In 1989, neither parent was present in home ranges of five sons and seven daughters that bred in their first year, and with a threefold density increase in 1990, 63% of males and 46% of females became sexually mature in their first summer if neither parent was present in their home range (Fig. 1). In the presence of fathers, 38% of sons became mature compared to 0% in the presence of mothers. Conversely, 67% of daughters became sexually mature in presence of mothers compared to 0% in presence of fathers. The number of juveniles that became sexually mature in presence of same-sex parents did not differ from when both parents were absent. Thus, reproductive suppression of juveniles of both sexes occurred in the presence of parents of the opposite, but not of the same sex. Pregnant or lactating females were found together in the same nest boxes on 18 occasions; on all occasions they were known to be sisters or mothers and daughters.

To determine whether juvenile dispersal from the natal site was inhibited by the presence of resident adults, I removed ~50% of the adult *Peromyscus* from one grid (removal grid) during 2-week trapping periods throughout June-October 1990. The number of new immigrant animals marked on this grid every 2 weeks averaged 39%, compared to 14% on control grids ($\chi^2 = 179.5$, $P < 0.001$; Fig. 2). Immigration was rapid and the population density on the removal grid was the same as that on

TABLE 1 Data from observed nest boxes

| | 1989 | 1990 | |
|---|---|---|---|
| | Control | Control | Removal |
| Number of mothers | 20 | 42 | 23 |
| Number of litters | 24 | 64 | 35 |
| Number of sons | 32 | 99 | 50 |
| remaining in natal home range ≥7 weeks | 5 (16%) | 49 (49%) | 21 (42%) |
| becoming sexually mature | 5 (100%) | 4 (8%) | 9 (43%) |
| Number of daughters | 31 | 94 | 40 |
| remaining in natal home range ≥7 weeks | 9 (29%) | 45 (48%) | 23 (57%) |
| reproducing | 7 (78%) | 14 (31%) | 8 (35%) |

Number of sons that became sexually mature and daughters that reproduced in natal home ranges on control and removal grids in 1989 and 1990. Sexual maturity (or reproductive condition) was defined for males as having large testes descended ino the scrotum, and for females as being pregnant or lactating[14,27]. Males were considered to have bred if they were found nesting with pregnant or lactating females[17].

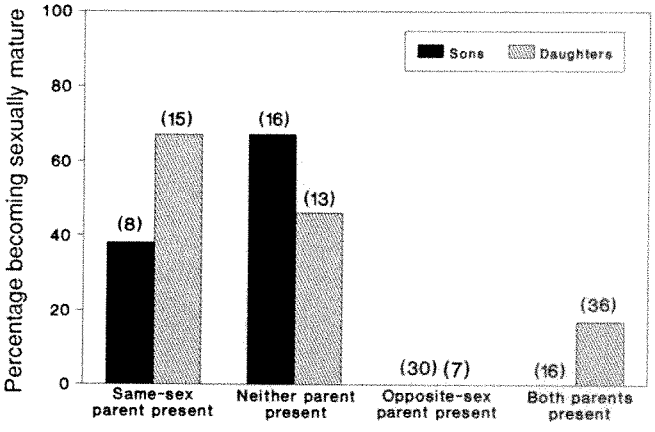


FIG. 1 The percentage of *P. Leucopus* sons and daughters that became sexually mature in the presence or absence of their mothers and fathers in the home range in 1990. Samples sizes are given above each bar. The number of offspring maturing in the presence of parents of the same sex did not differ significantly from the number of sons (Fisher's exact test, $P = 0.140$) or daughters ($\chi^2 = 1.343$, $P = 0.246$) maturing when neither parent was present. None of the 30 sons or 7 daughters became sexually mature in the presence of parents of the opposite sex, but 38% of 8 sons and 67% of 15 daughters matured with parents of the same sex; the differences in numbers becoming sexually mature with parents of the same and opposite sex were significantly different for both sons and daughters (Fisher's exact test, $P = 0.007$ and $P = 0.005$, respectively).

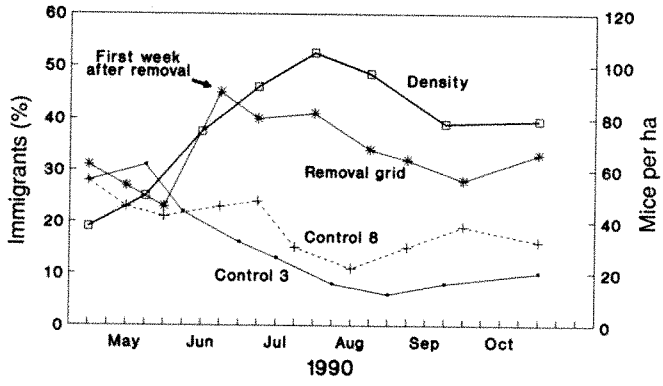


FIG. 2 The percentage of mice caught on one removal and two control grids during the 1990 high-density year that were new animals (presumed immigrants). Population density is the mean density on the two control grids.

control grids by the next trapping period. On the removal grid, 43% of juvenile males became sexually mature, compared to 8% on control grids ($\chi^2 = 9.519$, $P = 0.002$; Table 1). With almost 50% of the space being made vacant every two weeks, juvenile males apparently separated from their mothers and associated with non-relative females.

To determine whether the presence of parents of the opposite sex were the stimulus for sex-biased dispersal of juveniles, in 1991 I removed mothers from eight family groups and fathers from eight other family groups when the pups were 25–30 days old (weanlings). Sons remained significantly longer on natal home ranges from which mothers were removed than on those inhabited by mothers (medians 8.5 and 2 weeks, respectively; Wilcoxon medium test statistic $= -3.386$, $n = 26$, $P = 0.0007$), and conversely, daughters remained longer on natal home ranges from which fathers had been removed than on those inhabited by fathers (medians 7 and 2 weeks, respectively; Wilcoxon median test statistic $= -1.824$, $n = 22$, $P = 0.068$). The median residency for juveniles when both parents were present was two weeks, the same as when opposite-sex parents were present. I was unable to follow animals into the next breeding season; however, 8 of 14 sons remained for $\geq 7$ weeks on home ranges from which mothers were removed, whereas none of 12 sons remained on home ranges inhabited by mothers (Fisher's exact test, $P = 0.002$). Also, 8 of 13 daughters remained for $\geq 7$ weeks on the home ranges from which fathers were removed, whereas two of nine daughters remained on home ranges inhabited by fathers ($P = 0.099$). This confirmed previous observations that offspring tend toward philopatry when the parent of opposite sex is absent.

In conclusion, reproductive suppression results when juveniles are unable to separate from the parent of opposite sex. When population density is low, juvenile white-footed mice disperse to vacant habitats near their natal home ranges[6], whereas they are apparently inhibited from dispersing when density is high, perhaps because the habitat is saturated with aggressive territoral neighbours that prevent immigration[19]. The consequence of this 'social fence'[20] is that juveniles remain philopatric and are reproductively suppressed if opposite-sex parents are present. Residency of adults and juveniles increases at high densities, and adults are not aggressive towards juveniles of the same sex[16]; thus sex-biased dispersal and reproductive suppression do not result from competition with same-sex parents. The presence of extended families of mothers and daughters raising litters together also indicates that resource competition and suppression of reproduction in juvenile females by their mothers and siblings are unlikely. My findings support the inbreeding-avoidance hypothesis for sex-biased dispersal[6–10,21–26]. Any population regulation resulting from this reproductive suppression is possibly an artefact of daughters being unable to separate from their fathers. □

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# No evidence for variable duration of sympatry between the great spotted cuckoo and its magpie host

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**BROOD parasites and their hosts are thought to engage in coevolutionary arms race in which parasitism selects for adapti defences by the host (such as egg rejection), which in turn select for counter-adaptations by the parasite (such as egg mimicry)[1,2]. Soler and Møller have tested whether the duration of coevolution (measured by the duration of sympatry at three different geographic areas) in a host–cuckoo system affected egg-rejection behaviour by hosts[3]. They found that the extent of both rejection and recognition of parasitic eggs covaried positively with the duration of sympatry. Here we show that, in the absence of strong historical evidence, field data do not support the existence of variations in the duration of sympatry at the two areas where the distributional ranges of the cuckoo and its hosts overlap. The reported differences in egg rejection by hosts might alternatively reflect flexible behavioural responses to the presence of the adult parasite near the nest.**

Brood parasitic birds are becoming favoured examples in studies of coevolution[2]. Experiment has shown that mimicry in cuckoo eggs has evolved as a counter-adaptation to egg discrimination by hosts, which in turn evolved as a behavioural defence against parasitism. Some have tested host responses towards real or model cuckoo eggs with different degrees of mimicry in areas where the host is sympatric with the parasite and in areas where the host has (presumably) never been parasitized. This work found that rejection rates were lower by hosts in

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TABLE 1 Reproductive parameters of great spotted cuckoos in Guadix and Santa Fe

| | This study | | | Soler and Møller[3,8] | | |
|---|---|---|---|---|---|---|
| | Guadix | Santa Fe | *P* | Guadix | Santa Fe | *P* |
| Nests parasitized* (*n*) | 30.8 (130) | 60.7 (242) | <0.001 | 42.0 (57) | 50 (12) | n.s. |
| Nests containing more than 2 cuckoo eggs* (*n*) | 20.0 (13) | 38.0 (242) | <0.001 | | | |
| Parasitized nests with more than 2 cuckoo eggs* (*n*) | 65.0 (40) | 62.6 (147) | n.s. | 73.9 (23) | <73.9 | |
| Nests with cuckoo eggs laid by more than one female* (*n*) | 4.6 (130) | 16.9 (242) | <0.001 | | | |
| Parasitized nests with cuckoo eggs laid by more than one female* (*n*) | 15.0 (40) | 27.9 (147) | 0.07 | 21.4 (28) | | |
| Nests with multiple parasitism with more than one egg of the same female* (*n*) | 53.8 (26) | 83.1 (83) | <0.01 | 50.0 (18) | <50.0 | |
| Cuckoo eggs per nest (mean ± s.e.) (*n*) | 2.02 ± 0.17 (40) | 2.44 ± 0.12 (143) | n.s. | 2.20 ± 0.21 (23) | 1.80 ± 0.49 (5) | n.s. |
| Female cuckoos per nest (mean ± s.e.) (*n*) | 1.15 ± 0.06 (39) | 1.45 ± 0.05 (134) | <0.01 | | | |
| Fledgling cuckoos per nest (mean ± s.e.) (*n*) | 0.88 ± 0.15 (41) | 1.34 ± 0.11 (127) | <0.05 | | | |
| Cuckoo eggs that fledged* (*n*) | 43.2 (81) | 54.9 (295) | <0.05 | | | |

Nests were checked 2–6 times a week. Four clutches where we suspected that hosts had ejected cuckoo eggs before we detected them (as judged by the damage of host eggs[8]) were excluded from calculations of the number of cuckoo eggs per nest. The number of cuckoo females laying in each host nest was determined on the basis of egg characteristics and laying dates[8]. Ten clutches where the number of females could not be accurately determined were excluded from analyses. *n*, Number of nests; *P*, two-tailed probability levels in the comparison of proportions (Fisher's test) or means (Mann–Whitney test) between areas within studies; n.s., not significant.

* Values shown are percentages.

allopatry[3,4]. In addition, hosts in sympatry were less likely to reject a mimetic model egg than a non-mimetic one[3–7].

Recently Soler and Møller[3] provided a putative example of coevolution in action. They claimed that rejection of eggs of the great spotted cuckoo *Clamator glandarius* by magpie *Pica pica* hosts varied according to the duration of sympatry. In a population of presumed ancient sympatry (in Santa Fe, Spain), magpies readily rejected both mimetic and non-mimetic eggs; in an area of presumed recent sympatry (Guadix, Spain), 60 km away from Santa Fe, magpies were less discriminating, particularly of mimetic eggs; and in allopatry (Uppsala, Sweden), magpies showed no rejection at all. The key comparison here involves the areas of sympatry, which allows us to catch a glimpse of the dynamics of evolution and to test the view that cuckoos and hosts are engaged in a coevolutionary arms race, with cuckoos being one step ahead in areas of recent sympatry.

Soler argued that the higher altitude of the Guadix area (900–1100 m above sea level) had prevented cuckoos from colonizing it in the past, as the species seems to favour lowland below 500 m[8] (but see ref. 9). However cuckoos are very abundant in the Santa Fe area, which is 700–800 m above sea level. We collected data on cuckoo parasitism from the Guadix area during 1982–1984, and from the Santa Fe area during 1985 and 1989–1991. Despite its altitude, local parasitization rates at Santa Fe were high (Table 1), actually among the highest ever recorded for any species of parasitic cuckoo[2].

In the absence of detailed historical information other than a claim made by local hunters that great spotted cuckoos were absent from Guadix until 1962 (ref. 8), Soler and Møller assumed that cuckoos had only recently colonized the Guadix area. Apparently, this conclusion is based on the fact that cuckoos were expanding their host niche and exploiting magpie hosts more successfully in the area of recent sympatry. They argued in favour of this possibility on the following grounds[3,8]: (1) cuckoos "regularly parasitized three other corvid species in the Guadix area, whereas the magpie was the only commonly parasitized species in nearby areas of ancient sympatry, although all corvid species bred in both areas of recent and of ancient sympatry"[3]. This statement is erroneous. No study has been conducted in other areas in Spain where the cuckoo is sympatric with several potential hosts, and no corvids other than magpies have been observed to breed at Sante Fe. In fact, when describing the Santa Fe area, Soler[8] explicitly states that "only magpies nest in this area". (2) "The number of great spotted cuckoos laying in each host nest was larger at Guadix than in other areas"[3]. Although Soler[8] did not provide any comparative evidence, our data show the opposite pattern. The number of female cuckoos laying in each nest, and the proportion of nests containing eggs from more than one female, were both larger at Santa Fe (Table 1). (3) In the putatively recently colonized area, "individual female great spotted cuckoos frequently laid more than one egg in each host nest"[3]. Our estimate of the percentage of multiparasitized nests containing more than one egg of the same female at Guadix is close to that given by Soler[8], but significantly lower than that obtained at Santa Fe (Table 1). (4) "The three [above] behavioural changes among great spotted cuckoos in the areas of recent sympatry all led to increased reproductive success of the parasite"[3]. As above, no comparative data were given[8] but we found that cuckoos fledged more chicks both per nest and per egg laid at Santa Fe than at Guadix (Table 1).

Independently of whether such arguments have any bearing on the duration of sympatry between cuckoos and magpies, it is clear that with the larger sample sizes of our data, many of the trends oppose those claimed by Soler and Møller[3]. Even if Guadix is a recently occupied area, our data show that the supposed characteristics of a recent area do not apply. In any case, the differences between the two areas are probably unrelated to duration of sympatry. The occurrence of great spotted cuckoo parasitism on magpies was reported at Iznalloz (800–1,400 m above sea level, 40 km away from Guadix) as early as 1885 (ref. 10). Differences between the two areas may simply reflect the fact that the density of cuckoos relative to that of their hosts is larger at Santa Fe than at Guadix (Table 1).

This could in turn explain why a higher proportion of experimental eggs were rejected at Santa Fe than at Guadix, because hosts are known to be more likely to reject alien eggs when they are alerted by encounters with the adult parasite in the vicinity of their nest[1,7]. Phenotypic flexibility might thus provide an alternative explanation for relatively low rates of rejection in allopatry found in other studies[4,11,12]. □

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# Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*

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**INHERITANCE of mitochondrial DNA in animals was thought to be strictly maternal[1,2]. Recently, evidence for incidental paternal mtDNA leakage was obtained in hybrid crosses of *Drosophila*[3,4] and mice[5]. In mice, the frequency of paternal mtDNA contributions was estimated at $10^{-4}$, compared with maternal contributions. The common occurrence in the marine mussel *Mytilus* of heteroplasmic individuals with two or more types of highly diverged mtDNA molecules was interpreted as strong evidence for biparental mtDNA inheritance by some[6], but not by others[7]. We report here results from pair-matings involving two species of mussels, *Mytilus edulis* and *Mytilus trossulus*. Extensive contribution of paternal mtDNA, amounting to several orders of magnitude higher than that inferred for *Drosophila* or mice, was observed in both intra- and interspecific crosses.**

Mussels were collected from a mussel farm southwest of Halifax, Nova Scotia, Canada, classified provisionally as *M. edulis* or *M. trossulus* on the basis of shell morphology[8] and held in ambient running sea water. A day later several specimens of each species were individually spawned, and sperm and eggs from a designated pair were combined in one container to establish a family. Spawning and raising were done in sea water heated to 20 °C, filtered to 1 μm, and sterilized by ultraviolet light. Parents were frozen at −70 °C, and offspring were raised as described[9] and subsequently processed at 12–15 months of age for mtDNA and allozyme genotyping.

MtDNA profiles were scored after hybridization of total DNA digests to a probe containing equal amounts of three cloned fragments of the *M. edulis* and two cloned fragments of the *M. trossulus* mtDNA (Fig. 1). The mixed probe was used to avoid problems of differential band intensity due to heterologous hybridization. An independently derived set of clones covering the entire *M. edulis* mtDNA and used to sequence 13.5 kilobases (kb) of it[10] was used to verify parental patterns. They produced the same patterns, except that it was easier to detect small (<700 base pairs (bp)) *Hin*dIII fragments. But *M. trossulus* bands were generally fainter and some did not react at all.

Allozyme scoring was used to verify the species identity of parents and also to ensure that offspring with the male's mtDNA

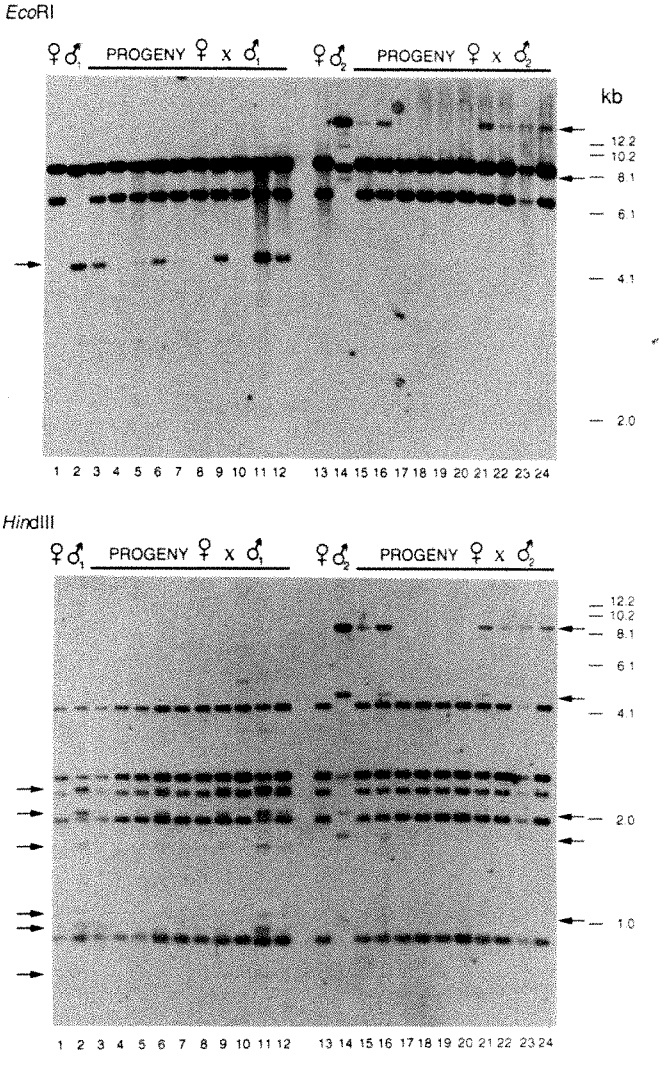


FIG. 1 Evidence of mtDNA biparental inheritance in two *Mytilus* families sharing the same female parent (EF16). Male 1 is EM15 (family 16, a homospecific family) and male 2 is TM15 (family 15, a heterospecific family). Restriction profiles of 10 offspring are shown from each family. Arrows point to the position of diagnostic paternal bands. Numbers at the right margin give lengths in kb. The heteroplasmy of male 1 can be seen in the *Hin*dIII profile. Male 2 has a large mtDNA (>22 kb). The minor *Eco*RI bands did not interact with *M. edulis* probes, suggesting they may contain tandemly repeated sequences. Corresponding bands are also visible in longer exposures of *Hin*dIII digests. Seven offspring from the first family (lanes 3, 5–7, 9, 11 and 12) and six from the second (lanes 15, 16, 21–24) carry paternal mtDNA. The faint upper bands in lanes 10 and 11 are apparently due to partial digestion of mtDNA, as their sizes match the sum of the two adjacent smaller bands.

METHODS. DNA was extracted from the gill and gonad of the parents and from most of the soft tissue of the offspring using a variation of a salt extraction procedure[17]. EDTA in the lysis buffer was increased to 10 mM, and the DNA in the supernatant, after the salt extraction, was extracted once with chloroform before ethanol precipitation. Total DNA (2 μg) was digested with 20 units of *Eco*RI or *Hin*dIII, separated on 0.8% agarose gels, and blotted to Amersham Hybond nylon using a Pharmacia transfer apparatus. A probe consisting of three *M. edulis* and two *M. trossulus* mtDNA clones was used for hybridization. The *M. edulis* clones were isolated from the combined mtDNA of two individuals after fractionation of total DNA on a caesium chloride gradient[18]. One of these individuals had the *Eco*RI pattern FB (female B) and the other the *Eco*RI pattern M previously reported[7]. Two of the cloned fragments were of about the same size (~8.6 kb), but had different *Xba*I and *Hin*dIII restriction profiles. Thus, one corresponds to the largest fragment of the FB pattern and the other to the largest fragment of the M pattern. The third cloned fragment was 4.1 kb, corresponding to one of the two comigrating fragments of pattern M[7]. The two *M. trossulus* clones (~13 and 7 kb) were isolated from purified mitochondria[19]. The clones were random primer labelled[20,21] with digoxigenin-labelled dUTP (dig-dUTP; Boehringer) and hybridized to the blots in Westneat buffer[22] at 58 °C. The final wash was 15 min at 58 °C in 0.2 × SSC, 0.2% SDS. The dig-dUTP was detected by an anti-dig/AMPPD assay according to the manufacturer.

TABLE 1 MtDNA restriction patterns of parents and numbers of offspring with paternal mtDNA in families of *Mytilus*

| Family | Female* | mtDNA profiles† *Eco*RI | *Hin*dIII | Male | mtDNA profiles *Eco*RI | *Hin*dIII | Offspring examined‡ | Contaminants§ | Positive/total scored‖ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | EF1 | FB | B | EM1 | M/FB | D/B | 9 | 1 | 0/8 |
| 2 | EF3 | FB | B | TM3 | S/N | U/N | 20 | 2 | 0/18 |
| 3 | EF4 | FB | A | EM4 | M/FB | D/B | 94 | 1 | 0/88 |
| 4 | TF4 | Q | Q | EM4 | M/FB | D/B | 53 | 19 | 12/28 |
| 5 | TF4 | Q | Q | TM4 | S/P | U/P | 48 | 20 | 14/25 |
| 6 | EF5 | FB | C | EM5 | FB/D | B | 10 | 1 | 3/3 |
| 7 | EF7 | C | B | EM7 | M/FB | E/B | 59 | 4 | 25/34 |
| 8 | EF7 | C | B | TM7 | O | Q | 13 | 2 | 2/6 |
| 9 | EF11 | FB | B | EM11 | M/FB | D/B | 10 | 1 | 7/9 |
| 10 | EF11 | FB | B | TM11 | O | Q | 10 | 2 | 4/7 |
| 11 | EF12 | FB | B | TM12 | D/FB | S/A | 10 | 2 | 0/7 |
| 12 | TF13 | R | R | TM13 | N/S | O | 10 | 2 | 0/8 |
| 13 | TF14 | N | N | TM14 | N/S | N/T | 10 | 3 | 3/7 |
| 14 | EF15 | FB | B | TM15 | T/O | P/Q | 49 | 7 | 17/35 |
| 15 | EF16 | FB | A | TM15 | T/O | P/Q | 58 | 1 | 39/45 |
| 16 | EF16 | FB | A | EM15 | M/FA | D/A | 52 | 1 | 23/40 |
| Total | | | | | | | 515 | 69 | 149/368 |

* Individuals are identified by E (*M. edulis*) or T (*M. trossulus*), by F (female) or M (male), and by a number. They were classified as *M. edulis* or *M. trossulus* on the basis of phosphomannose isomerase (PMI, EC 5.3.1.8) which is species diagnostic[1,3]. TM7 was an allozyme hybrid but had the *M. trossulus* mtDNA.

† Two *Eco*RI or *Hin*dIII patterns (separated by a slash) are given for heteroplasmic males, with the first pattern predominating. *Eco*RI patterns FB, FA and M were described previously[7]. *M. trossulus* patterns *Eco*RI P, Q and R and *Hin*dIII P, Q and R differ by the position of one size-variable band.

‡ Includes offspring scored for one or two restriction enzymes and for allozymes. The enzyme loci PMI, octopine dehydrogenase (EC 1.5.1.11), phosphoglucose isomerase (EC 5.3.1.9), phosphoglucomutase (EC 5.4.2.2) and 6-phosphogluconic dehydrogenase (EC 1.1.1.44) were used either because they were diagnostic for the two species or because they were highly polymorphic.

§ Includes offspring whose allozyme genotype for one or more loci is incompatible with parental genotypes, or whose mtDNA pattern for one or both restriction enzymes is incompatible with the maternal pattern. If contamination was due to inadvertent transfer of larvae from one container to another, the possibility of not detecting a contaminant can be firmly excluded. Pairwise comparisons of families showed that in all pairs but one (7 and 8), contaminants can be identified on the basis of allozymes or maternal mtDNA, or both. If a contaminant entered by sea water, the probability that it would escape detection can be calculated if we assume that maternal mitotype and allozyme frequencies among parents are reasonable estimates of those in the population. The probability varies among families from 0.0001 to 0.02. If contamination was due to females having collected sperm before spawning, the probability of not detecting a contaminant depends only on the male's allozymes in the sperm pool and varies from 0.016 to 0.251. The probability that an undetected contaminant also happened to carry the male's mtDNA is the product of the probability of not being detected and the frequency of male's mtDNA in the population (these frequencies can be estimated from the male mitotypes counting both types of heteroplasmic males) and varies from less than 0.0001 to 0.028 depending on the family and on whether contamination was by sea water or sperm retention. Finally, the probability that more than one contaminant of this type will be found in the same container is the above probability raised to the number of offspring with paternal mtDNA. It is much less than 0.0001 in all 11 families with paternal inheritance.

‖ The numerator is the number of offspring with paternal mtDNA. Only compatible offspring scored for both restriction enzymes are included in the denominator.

were sired by that male. MtDNA and allozyme genotyping revealed that some juveniles did not belong to the family to which they were assigned, forcing a classification of offspring into 'compatible' and 'contaminant'. The most likely explanation of contamination is that bucket washing or aeration (air bubbling caused larvae to become airborne and land in an adjacent container) resulted in inadvertent transfer of larvae from one container to another. The possibility that larvae or gametes entered from the wild through the pumped sea water can be dismissed given the treatment of the water. Females could have collected and retained sperm in their mantle cavities while in the wild or in the holding tank before spawning. Under any of these circumstances, the probability that a contaminant will be compatible with the allozymes and the maternal mtDNA of the family and, in addition, will carry the mtDNA type of the family's male, is very small. The probability that several such contaminants will be found in the same family is even smaller (Table 1).

Seventeen composite (two-enzyme) restriction patterns were observed among the 26 adults used as parents (Table 1). The previously described *EFB* and *EM Eco*RI patterns[7] were the most common among *M. edulis* females and males, respectively, and heteroplasmy was more common among males, as observed previously[7]. No common patterns were seen in the two species. *M. trossulus* mtDNA profiles frequently summed to a total of 22–25 kb, and consisted of size-invariable and size-variable bands. This type of variation is common in scallops because of varying copy-number of species-specific tandemly repeated sequences[11]. The presence of such sequences in *M. trossulus* mtDNA may also explain the poor reaction of the size-variable bands to *M. edulis* probes.

Studies of paternal mtDNA inheritance must address two opposing methodological difficulties[1,2,7]: (1) offspring scored as not having the male's mtDNA may have it in amounts undetectable by the method used; and (2) if offspring were found to contain the male's mtDNA type, it can always be claimed that the mother was heteroplasmic for this mtDNA type in amounts beyond the detection power of the method used and this type was amplified in the offspring.

The first difficulty helps to highlight our finding of a high rate of paternal mtDNA inheritance in mussels. From a dilution series experiment we estimated that we could detect minority mtDNA bands present in ratios of 1/1,000. Under these conditions 11 of the 16 families produced direct evidence of biparental inheritance with an overall rate of 40% (149/368, Table 1). Clearly, these are minimum estimates. Stochastic multiplication of different mtDNA molecules during development[12] is expected to amplify variation in the sperm's contribution and result in large differences in the amount of paternal mtDNA among offspring, as observed. It is not clear if this could explain the apparent absence of paternal mtDNA in some families, particularly in family 3 where no such DNA was detected. Maternal control, nuclear or cytoplasmic, of sperm mtDNA rejection is a possibility.

For our study, the second methodological difficulty is more important. We have overcome it by mating the same female with

two males having different mtDNA restriction profiles. Among these four pairs of families we have observed no offspring with diagnostic bands of the male that sired the other family (Fig. 1). This also eliminates the possibility that contaminants from unrelated heteroplasmic females caused a false impression of biparental inheritance, as it would imply two types of contaminants each found only in the family whose male had the matching mtDNA pattern.

We also observed biparental inheritance in intraspecific crosses. Both in *Drosophila* and in mice the crosses used to show paternal mtDNA contribution were between sibling species. *M. edulis* and *M. trossulus* have been recognized as different taxa on the basis of shell morphology and allozyme frequencies[8] (mtDNA differences reported here corroborate the distinction). Recent studies[13,14] did not recognize further subdivisions of either species.

This is the first direct observation of paternal mtDNA transmission over the span of one generation (both in *Drosophila* and in mice the paternal mtDNA type was detected after multiple backcrossing to a paternal strain[3-5]). Assuming that about a quarter of mtDNA molecules in 40% of offspring were of paternal origin provides a conservative estimate of paternal contribution of 0.1 which is 1,000 times larger than that for mice[5]. The finding confirms the suggestion of Hoeh *et al.*[6] that biparental mtDNA inheritance is common in the wild populations of mussels and is probably the main cause of the high incidence of heteroplasmy seen in this genus (refs 6, 7 and this study). Indirect support of paternal transmission of mitochondria in *Mytilus* comes from morphological studies of spermatogenesis and fertilization of this species[15]. There is insufficient information to determine whether the high level of paternal mtDNA transmission we have observed is peculiar to *Mytilus*. Paternal mtDNA transmission of some degree occurs in *Drosophila*, mussels and mice and was also inferred in anchovies on the basis of heteroplasmy for highly diverged molecules[16]. This diverse collection of animals suggests that the phenomenon might be widespread, with obvious implications for the use of the mtDNA molecule for population and phylogenetic studies. □

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# Parental origin of chromosomes involved in the translocation t(9;22)

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**FUNCTIONALLY equivalent genetic material can be labelled by an epigenetic marking process and used differentially depending on whether its origin is maternal or paternal[1]. This phenomenon is known as genomic imprinting and is manifested at either the chromosomal or gene level. Genomic imprinting seems to play an important role in cancer predisposition syndromes[2-5], and phenotypic consequences are evident in constitutional deletion syndromes and uniparental disomies[1]. Moreover, there seems to be a preferential retention of paternal alleles in sporadic tumours such as Wilms' tumour[6], rhabdomyosarcoma[7], osteosarcoma[8] and retinoblastoma[9]. To investigate whether chromosomes involved in acquired abnormalities of haematologic neoplasms show a similar 'parent of origin' bias, we studied the inheritance of the translocated chromosomes 9 and 22 in cases of Philadelphia-chromosome-positive leukaemia, using unique specific chromosome band polymorphisms. Here we show that the translocated chromosome 9 was of paternal origin, whereas the translocated chromosomes 22 were derived exclusively from the maternal copy, in 11 cases with reliable polymorphisms. Our data therefore provide evidence that imprinting phenomena may play an important role in acquired tumour-specific chromosome rearrangements.**

Both cytogenetic and molecular genetic methods are used to analyse the parental origin of numerical and structural chromosome abnormalities[10-13]. Molecular techniques are preferable in the determination of heterozygosity at polymorphic loci and are often the only way to study quantitative deviations and submicroscopic mutations. But these approaches are not readily able to distinguish between paternal and maternal homologues contributing to balanced rearrangements. As these do not involve quantitative deviations, molecular genetic analysis would be feasible only in cases with intragenic polymorphisms which, in addition, would have to be altered by the respective rearrangement. Alternatively, homologous chromosomes would have to be separated by generating cell hybrids or by microdissection before analysis for molecular polymorphisms. Cytogenetic analysis of chromosomes with banding polymorphisms allows determination of the parental origin of the chromosomes[11,13]. To investigate the origin of chromosomes contributing to the leukaemia-specific reciprocal translocation t(9;22)(q34;q11), we have analysed the C-band polymorphisms of the heterochromatin regions on chromosomes 9 and the silver-staining pattern polymorphisms of the nucleolus-organizing region (Ag-NOR) of chromosomes 22 in 15 patients (Table 1). Within the human karyotype, C-bands that are inherited in a mendelian fashion are located at the centromeres of all chromosomes, at the secondary constrictions of chromosomes 1, 9 and 16 and at the long arm of the Y chromosome. These regions vary extensively in size, and may be located at the long and/or short arm of the chromosomes. In a normal population, the two homologous chromosomes 9 can be distinguished by their heterochromatin polymorphism in about 80% of individuals, thus facilitating pedigree studies[14]. The short arms, the satellites and the satellite stalks of chromosome 22 also vary in size and are highly polymorphic as shown by several staining procedures[13-15]. Nucleolar organizing regions are localized in the secondary constriction region on the short arm of acrocentric chromosomes, including chromosome 22. These regions can be specifically stained with silver and appear as dot-like structures. Although a particular Ag-NOR staining pattern is a stable and inherited property, it may be affected by technical variations[15].

These patterns are used to identify the parental origin in human trisomies of acrocentric chromosomes[13].

Our results demonstrate that the rearrangement t(9;22) affected the paternal chromosome 9 and the maternal chromosome 22 in a nonrandom manner (Table 1; Figs 1 and 2), regardless of the age or sex of the patients or the underlying type of leukaemia and gene rearrangement (Table 1). Two mechanisms may account for the observed distribution of paternally and maternally derived chromosomes. If both copies of chromosomes 9 and 22 had an equal chance of being translocated, the observed pattern would result from selective expansion of a clone with the translocation combining these particular paternal and/or maternal chromosomes. Alternatively, certain regions on one of the homologous chromosomes could be more susceptible to rearrangements.

The latter model can be explained by the presence of epigenetic modifications such as DNA methylation, because methylated DNA is inherently susceptible to certain types of mutations[16]. Allele-specific methylation may thus render the copy with the higher degree of methylation prone to a higher mutation risk[16]. Differentially methylated maternal and paternal chromosomes have been demonstrated in transgenic mice, thus supporting the notion that methylation represents a potential regulatory mechanism for genomic imprinting[17,18]. Alterations of the DNA methylation state are common in mammalian tumours[16,19], therefore defective methylation might promote instability in somatic chromosomes and thereby provide the basis for many of the chromosome abnormalities associated with cancer[20]. It is intriguing that genomic imprinting has been demonstrated for mouse chromosome areas homologous to regions on the long arms of human chromosomes 9, corresponding to mouse chromosome region 2A–C1 (T13H), and human chromosome 22, corresponding to mouse chromosome region 11A, involved in the Philadelphia (Ph)-translocation[1].

The two genes involved in the Ph-translocation, *abl* and *bcr*, are expressed in all normal tissues, human cell lines and leukaemic cell populations tested so far[21,22]. But it is not known whether the paternal, the maternal or both alleles are transcribed. The translocation t(9;22) results in a juxtaposition of the *abl* and *bcr* genes and generates a new hybrid gene that seems sufficient to confer the disease[21]. In addition, the chromatin conformation, the methylation status and the DNase I sensitivity

TABLE 1 Characteristics of 15 patients with Philadelphia-chromosome positive-leukaemias and parental origin of their translocated chromosomes 9 and 22

| Patient | Sex | Age | Disease | *bcr/abl* | der(9) | der(22) |
|---|---|---|---|---|---|---|
| 1 | M | 2 | CML | 5′ | PAT | MAT |
| 2 | M | 8 | CML | 3′ | PAT | MAT |
| 3 | F | 10 | CML | n.a. | PAT | MAT |
| 4 | F | 14 | CML | 5′ | n.c. | MAT |
| 5 | M | 16 | CML | 5′ | PAT | MAT |
| 6 | M | 20 | CML | n.a. | PAT | MAT |
| 7 | M | 20 | CML | n.a. | n.c. | MAT |
| 8 | F | 23 | CML | 5′ | n.c. | MAT |
| 9 | F | 25 | CML | n.a. | n.c. | n.c. |
| 10 | M | 42 | CML | n.a. | PAT | n.c. |
| 11 | F | 48 | CML | 5′ | PAT | MAT |
| 12 | F | 49 | CML | 5′ | PAT | MAT |
| 13 | F | 16 | ALL/CML | 5′ | PAT | n.c. |
| 14 | M | 7 | ALL | BCR1/ABL | PAT | MAT |
| 15 | M | 14 | ALL | 3′ | PAT | n.c. |

Twelve patients suffered from chronic myeloid leukaemia (CML) and two from acute lymphoblastic leukaemia (ALL). Patient 13 was diagnosed and treated as ALL, but developed CML after cessation of ALL therapy. Patient 7 had a spinal tumour at diagnosis which was interpreted as a localized primary lymphoblastic CML blast crisis. Patient 15 developed Ph-chromosome-positive ALL, 10 years after successful treatment of Ph-negative ALL. Except for patient 12, who had an additional complex t(7; 16; 17), all patients had the typical t(9; 22)(q34; q11) with no further karyotype changes at the time of analysis. Gene rearrangements (*bcr/abl*) were analysed in 10 cases by polymerase chain reaction as described[31]. The parental origin of the translocated chromosomes 9 was determined cytogenetically with C-band polymorphisms. The translocated chromosome 9 was inherited from the father in 11/11 cases in which its origin could be firmly established. In contrast, there was no evidence for a maternally derived translocated chromosome 9 in any of the cases investigated. The parental origin of the translocated chromosomes 22 was determined cytogenetically with silver staining polymorphisms of the nucleolus organizing region (Ag-NOR) located on the short arm. The maternal origin of the chromosome 22 affected by the translocation was detectable in 11 instances. Moreover, there was no indication for a paternal origin of any translocated chromosome 22 (see legend to Figs 1 and 2 for details). M, male; F, female; 3′, *bcr* exon 2/*abl* exon 2; 5′, *bcr* exon 3/*abl* exon 2; *BCR1/ABL*, *bcr* exon 1/*abl* exon 2; n.a. not analysed; der(9), translocated chromosome 9; der(22) translocated chromosome 22; PAT, paternally derived chromosome; MAT, maternally derived chromosome; n.c. not conclusive.

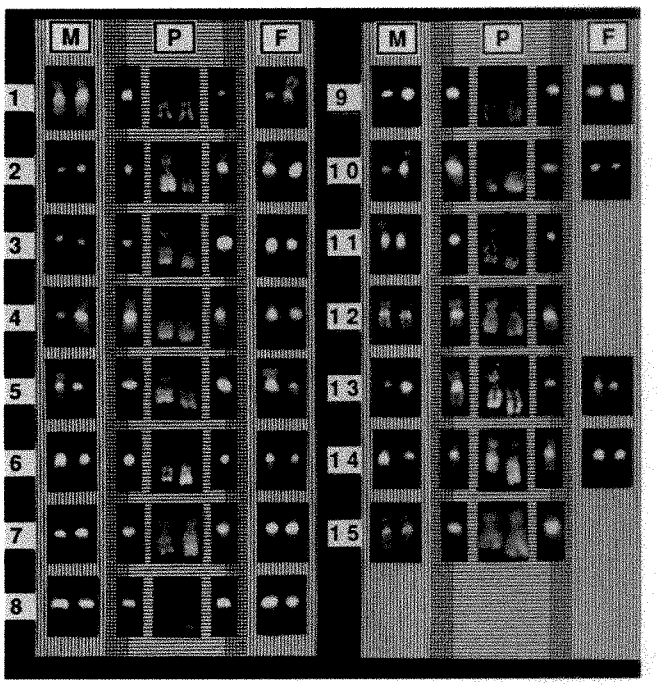


FIG. 1 Normal and translocated chromosomes 9 from 15 patients with Ph-positive leukaemia compared with the corresponding maternal and paternal chromosomes 9. Chromosomes were prepared according to standard techniques. Slides were R- and C-banded simultaneously with chromomycin A3 and distamycin A/DAPI (ref. 32) and karyotyping was done on a Genevision 121 chromosome analysis system. At least 50 metaphases were analysed in each patient and at least 80 metaphases in each parent. The parental origin of the patients' normal and translocated chromosomes 9 was determined by the inherited polymorphic size of the centromeric heterochromatin region which can be visualized by C-banding. C-banded maternal chromosomes 9 are shown in column M and the paternal ones in column F (vertical lined background). The patients' chromosomes are shown in column P with the corresponding paternally and maternally derived C-banded chromosomes on the checked background. Simultaneous R-banding of the same chromosomes (horizonal lined background) identified the translocated chromosome 9 (right copy). In three cases (11, 12 and 15), the father was not available.

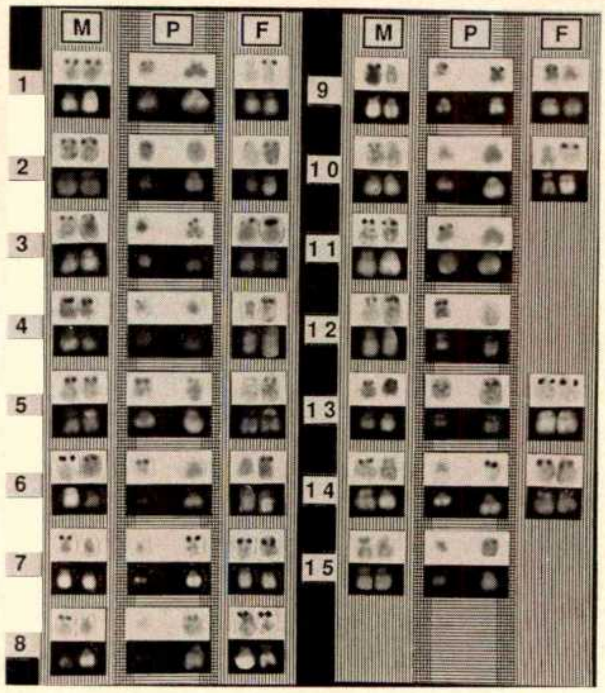


FIG. 2 Normal and translocated chromosomes 22 from 15 patients with Ph-positive leukaemia compared with the corresponding maternal (M) and paternal (F) chromosomes 22. R- and C-banded preparations were consecutively Ag-NOR-stained[33]. In each case, Ag-NOR-stained chromosomes are shown in the top row (white background) and, to verify that these were chromosomes 22, R-banding of the same chromosome pairs is shown (black background). Maternal chromosomes 22 are shown in column M and the paternal ones in column F (vertical lined background). The patients' normal (right) and translocated (left) chromosomes 22 are shown in column P (horizontal lined and checked background).

pattern at the translocation breakpoints differ from their normal alleles[23,24]. DNA methylation is involved in the multilevel control of eukaryotic gene expression[19] and in the stabilization of transcriptionally inactive chromatin domains[20]. In normal mammalian tissue, transcriptionally active or potentially active genes are hypomethylated and are hypersensitive to DNase I[25]. The 9q34 region is hypomethylated both in normal and in translocated chromosomes. The fact that the region 22q11, which is hypermethylated in both normal homologues, becomes hypomethylated after the translocation event, may therefore be related to acquired genetic activity at one or both translocation sites on chromosomes 9 and 22 (refs 23, 24). This observation fits a model for the development of Wilms' tumour: somatic crossing-over in a critical region of the affected chromosome results in the juxtaposition of two differently imprinted domains, thus exerting different 'position effects' on the adjacent genes depending on the domains involved[26]. Such position-effect variegation is also encountered during virus integration and insertion of transgenes into the genome[27,28]. Depending on the position and the genetic background, the penetration and expressivity of translocated or inserted foreign DNA can vary markedly[27-29]. Conversely, such events can alter the methylation status of the host DNA and, therefore, the activity of adjacent genes over a considerable distance[2,29]. Similarly, in the case of the Ph-translocation, expression of genes that are remote from the breakpoints, such as those for the immunoglobulin light chain and the $\beta$-chain of the platelet-derived growth factor on chromosome 22, may be modified. Altered expression of these genes might be involved in particular clinical and biological features of the disease, such as lymphoblastic transformation or thrombocytosis, and could therefore be of prognostic importance[23].

The cytogenetic markers used to assess the parental origin of the translocated chromosomes are distant from the breakpoints. The occurrence of somatic recombinations within these two regions cannot therefore be excluded. Mitotic recombination seems to be rare during normal mammalian development and is difficult to investigate[30]. But in patients with predisposing germ-line mutations, such events seem common in generating homozygous gene regions that are involved in tumorigenesis[3]. Accordingly, a translocation event can be considered as an illegitimate recombination between two nonhomologous chromosomes. The hypothetical occurrence of crossovers along the q-arms of the translocated and normal chromosomes 9 and 22 homologues, however, would provide support for exceptions to the rule rather than to the observed parent-of-origin bias of the chromosomes involved. We conclude that the preferential participation of the paternally derived chromosome 9 and the maternally derived chromosome 22 in the Ph-translocation strongly indicate that the chromosome regions involved may be imprinted. □

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# Synaptic vesicle-associated $Ca^{2+}$/calmodulin-dependent protein kinase II is a binding protein for synapsin I

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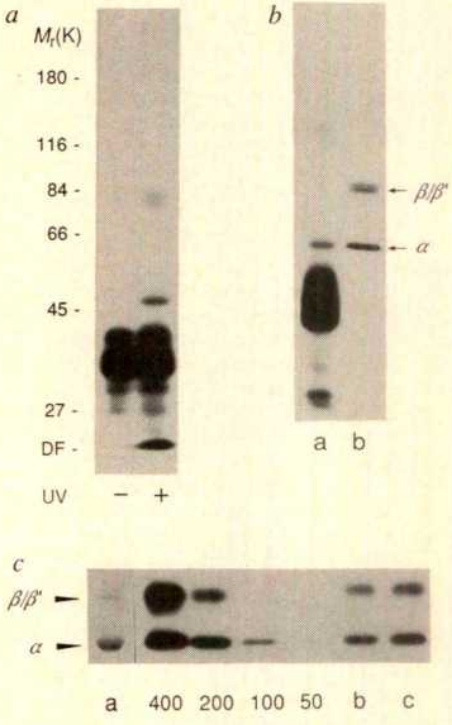
**SYNAPSIN I is a synaptic vesicle-associated phosphoprotein that is involved in the modulation of neurotransmitter release[1]. $Ca^{2+}$/calmodulin-dependent protein kinase II, which phosphorylates two sites in the carboxy-terminal region of synapsin I, causes synapsin I to dissociate from synaptic vesicles[2] and increases nerotransmitter release[3,4]. Conversely, the dephosphorylated form of synapsin I, but not the form phosphorylated by $Ca^{2+}$/calmodulin-dependent protein kinase II, inhibits neurotransmitter release[4-6]. The amino-terminal region of synapsin I interacts with membrane phospholipids, whereas the C-terminal region binds to a protein component of synaptic vesicles[7,8]. Here we demonstrate that the binding of the C-terminal region of synapsin I involves the regulatory domain of a synaptic vesicle-associated form of $Ca^{2+}$/calmodulin-dependent protein kinase II. Our results indicate that this form of the kinase functions both as a binding protein for synapsin I, and as an enzyme that phosphorylates synapsin I and promotes its dissociation from the vesicles.**

A synaptic vesicle component to which the C-terminal region of synapsin I binds was identified using the $^{125}$I-labelled cross-linking reagent sulphosuccinimidyl 2-(*p*-azidosalicylamido)-ethyl-1,3′-dithioproprionate (SASD)[9]. A protein of $M_r$ 48,000 was specifically labelled during incubation of the chemically modified C-terminal fragment of synapsin I with synapsin I-depleted synaptic vesicles (Fig. 1*a*). The $^{125}$I-labelled protein was extracted from preparative gels, and three peptides generated by proteolytic digestion were analysed by microsequencing. The sequences obtained for peptide 1 (DFPSPE; single-letter amino-acid code) and peptide 2 (DTVTPEAK) corresponded to residues 231–236 and 238–245, respectively, of the $\alpha$-subunit of rat brain $Ca^{2+}$/calmodulin-dependent protein kinase II ($Ca^{2+}$/CaM-dependent PK II). These sequences are present in the deduced primary structure of all five identified kinase subunits[10,11]. The sequence obtained for peptide 3 (DXKXQIVHFHRSGAPSXL) was specific for residues 459–476 of the $\alpha$-subunit of the kinase. Immunolabelling of blots from one-dimensional gels (Fig. 1*b*), using $Ca^{2+}$/CaM-dependent PK II-specific antibodies, revealed that both the $\alpha$- and $\beta/\beta'$ subunits of the enzyme were present on synaptic vesicles. But only the immunoreactive $\alpha$-subunit comigrated with the protein labelled with $^{125}$I after crosslinking.

Experiments using a variety of extraction procedures indicated that $Ca^{2+}$/CaM-dependent PK II was tightly associated with the vesicles, being solubilized only on treatment with non-ionic detergent (not shown). Quantitative analysis, using soluble $Ca^{2+}$/CaM-dependent PK II purified from rat forebrain as a standard (subunit ratio, $\alpha:\beta/\beta' = 3:1$), revealed that the vesicle-associated $Ca^{2+}$/CaM-dependent PK II subunits were present in a similar ratio (subunit ratio ±s.e.m., $2.81 \pm 0.21$; $n = 5$) and that they collectively represented $1.78 \pm 0.11$ and $1.91 \pm 0.15$% of the total protein for native and synapsin I-depleted synaptic vesicles, respectively (Fig. 1*c*). In the same




48K protein was eluted from the gel, acetone-precipitated and digested with endoproteinase Asp-N for 15 h at 37 °C. Peptides derived from the protease digestion were purified by reverse-phase HPLC (Vydac C-18 column). Sequencing was done with an Applied Biosystems gas-phase sequenator by the Rockefeller University Protein Sequencing Facility.

FIG. 1 The C-terminal fragment of synapsin I binds to a synaptic vesicle-associated form of $Ca^{2+}$/CaM-dependent PK II. *a*, Autoradiography of an SDS–9% polyacrylamide gel in which synaptic vesicle proteins were separated after photoaffinity labelling with the modified, $^{125}$I-labelled C-terminal fragment of synapsin I. On photolysis (UV), the appearance of a radiolabelled protein band of $M_r$ 48K was observed (right lane). The prominent 36–38K doublet present in both photolysed and non-photolysed samples corresponded to the vesicle-bound C-terminal fragment of synapsin I. The photolysis-dependent band observed at the bottom of the gel was due to low-level background radioactivity accumulating at the dye front (DF). *b*, A sample of synaptic vesicles photolysed after incubation with the modified, $^{125}$I-labelled C-terminal fragment of synapsin I was solubilized and the proteins were separated by SDS–PAGE and transferred to nitrocellulose membranes. The same sample was subjected sequentially to: a, autoradiography and b, immunolabelling with affinity-purified polyclonal antibodies that recognize both $\alpha$ and $\beta/\beta'$ subunits of $Ca^{2+}$/CaM-dependent PK II, followed by immunoperoxidase staining. *c*, (a) Coomassie blue staining of a sample of soluble $Ca^{2+}$/CaM-dependent PK II (3 μg) purified from rat forebrain[18]. Immunoblotting of native (b) and synapsin I-depleted synaptic vesicles (c) (10 μg protein) and of the indicated amounts (50–400 ng) of purified $Ca^{2+}$/CaM-dependent PK II are shown. An affinity-purified antibody recognizing the $\alpha$ and $\beta/\beta'$ subunits of $Ca^{2+}$/CaM-dependent PK II, followed by $^{125}$I-labelled-protein-A overlay, was used.

METHODS. The C-terminal fragments of synapsins Ia and Ib ($M_r$, 39–40 and 35–36K respectively, and collectively referred to as the C-terminal fragment of synapsin I) were generated by cysteine-specific cleavage and purified to homogeneity[19]. The fragment (0.8 mg ml$^{-1}$) was conjugated with 1.4 mM SASD[9] for 40 min at room temperature in the dark, in buffer (50 mM HEPES, pH 8.0, 100 mM NaCl). Iodination of the chemically modified fragment was carried out as described[20]. The modified, $^{125}$I-labelled C-terminal fragment (100–400 nM final concentration) was incubated for 30 min on ice in the dark, with synaptic vesicles (10–30 μg total protein) that had been previously depleted of the endogenous synapsin I by dilution in high salt medium[2,21]. After high-speed sedimentation, the synaptic vesicle pellets were photolysed for 2 min at 254 nm using an ultraviolet lamp (UVP, San Gabriel, California) and subjected to SDS–PAGE under reducing conditions[22]. The $^{125}$I-labelled

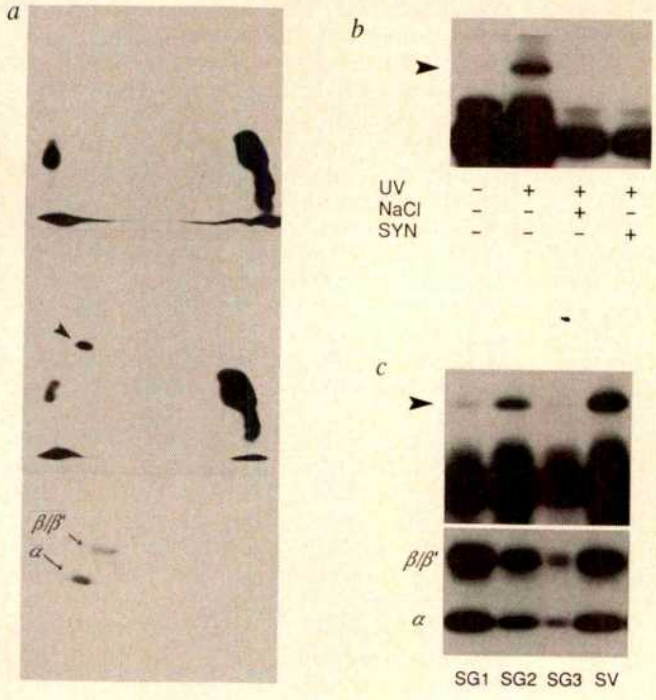



FIG. 2 Specificity of the interaction between synaptic vesicle-associated $Ca^{2+}$/CaM-dependent PK II and the C-terminal fragment of synapsin I. *a*, Autoradiography of two-dimensional polyacrylamide gels of samples where the modified, $^{125}$I-labelled C-terminal fragment of synapsin I was incubated in the absence (upper panel) or presence (middle panel) of synapsin I-depleted synaptic vesicles, and photolysed. Two-dimensional gel electrophoresis was done as described[23] in the presence of urea and Triton CF10 in the first dimension (from left to right) and on an SDS–9% polyacrylamide gel in the second dimension (from top to bottom). Proteins were electrotransferred from the two-dimensional gels to nitrocellulose filters for autoradiography and immunoblotting. Lower panel: immunoperoxidase staining of the sample shown in the middle panel, done using affinity-purified polyclonal antibodies that recognize both $\alpha$ and $\beta/\beta'$ subunits of $Ca^{2+}$/CaM-dependent PK II. *b*, Autoradiogram of an SDS–polyacrylamide gel in which synaptic vesicle proteins were separated after photoaffinity labelling (UV) with the modified C-terminal fragment of synapsin I in the presence of either 200 mM NaCl (NaCl) or a 10-fold excess of unlabelled synapsin I (SYN) as indicated. *c*, Upper panel: autoradiography of an SDS–9% polyacrylamide gel in which various rat brain membrane fractions (20 μg protein) obtained during the purification of synaptic vesicles[21] were separated, after photoaffinity labelling with the modified C-terminal fragment of synapsin I. Lower panel: the presence of $Ca^{2+}$/CaM-dependent PK II in the various membrane fractions (5 μg protein) is shown by immunoblotting. SG1, SG2 and SG3 correspond to fractions of light synaptosomal membranes separated by centrifugation through a 50–800 mM continuous sucrose density gradient. SG2 is the fraction specifically enriched in synaptic vesicles. SV designates the purified synaptic vesicle pool obtained after controlled-pore glass chromatography of the SG2 fraction[21]. Incubation and photolysis conditions as described in the legend to Fig. 1. Arrowheads indicate the position of the $^{125}$I-labelled $\alpha$ subunit of $Ca^{2+}$/CaM-dependent PK II.

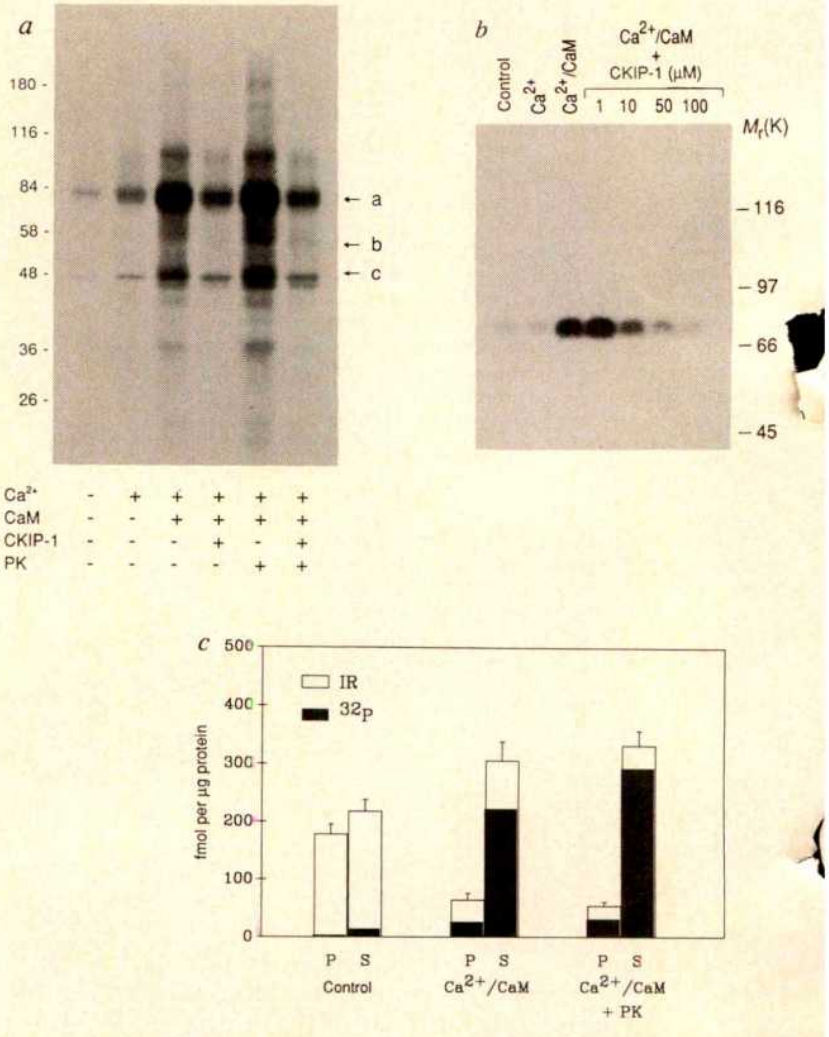



FIG. 3 Phosphorylation of synapsin I by synaptic vesicle-associated $Ca^{2+}$/CaM-dependent PK II. *a*, Endogenous $Ca^{2+}$/CaM-dependent phosphorylation of synaptic vesicle proteins. Purified synaptic vesicles were phosphorylated for 5 min at 30 °C in the absence or presence of 1 mM $Ca^{2+}$, 10 μg ml$^{-1}$ calmodulin (CaM) and 0.5 μg ml$^{-1}$ $Ca^{2+}$/CaM-dependent kinase II (PK), as indicated, under standard conditions (20 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 10–50 μM [$\gamma$-$^{32}$P]ATP (15–25 Ci mmol$^{-1}$)). In the samples where $Ca^{2+}$ was absent, 1 mM EGTA was added. The specificity of the phosphorylation reaction was assessed by adding 50 μM of the $Ca^{2+}$/CaM-dependent PK II inhibitory peptide CKIP-1, corresponding to residues 281–302 of the $\alpha$ subunit of the kinase, with Ala substituted for Thr at position 286. Phosphorylated proteins were analysed by SDS–PAGE[22] and autoradiography. The major phosphorylated bands (a, b and c in the figure) correspond to synapsin I and to the $\beta/\beta'$ and $\alpha$-subunits of $Ca^{2+}$/CaM-dependent PK II, respectively. *b*, Immunoprecipitation of synapsin I after endogenous phosphorylation by synaptic vesicle-associated $Ca^{2+}$/CaM-dependent PK II. Phosphorylation conditions were as described in the legend to Fig. 2*a*. The reaction was terminated by the addition of buffer for immunoprecipitation[24]. *c*, Regulation of the binding of the C-terminal fragment of synapsin I to synapsin I-depleted synaptic vesicles after phosphorylation by $Ca^{2+}$/CaM-dependent PK II. The unmodified C-terminal fragment of synapsin I was bound to synapsin I-depleted synaptic vesicles as described[7]. Phosphorylation conditions were as described in the legend to Fig. 2*a*, except that the $MgCl_2$ concentration was 1 mM. After phosphorylation, the amounts of the C-terminal fragment of synapsin I present in the high-speed pellet (P) and supernatant (S) fractions were analysed by quantitative immunoblotting (IR)[25]. Values expressed in fmol per μg protein are means (±s.e.m.) of five experiments. The $^{32}$P-incorporation into sites 2 and 3 of the C-terminal fragment of synapsin I was evaluated by liquid scintillation counting of the excised labelled bands and the values were normalized for the number of $Ca^{2+}$/CaM-dependent PK II sites (fmol $^{32}$P per μg protein per site).

preparations, synapsin I represented 6% of the total native synaptic vesicle protein. Therefore, synapsin I and $Ca^{2+}$/CaM-dependent PK II are present on the vesicle membrane in a molar ratio of about 2.6:1. This ratio indicates that each molecule of $Ca^{2+}$/CaM-dependent PK II can bind more than one molecule of synapsin I or that other vesicle proteins and/or phospholipids contribute to the binding of synapsin I to synaptic vesicles.

A variety of experimental data support the specificity of the interaction between the modified C-terminal fragment of synapsin I and synaptic vesicle-associated $Ca^{2+}$/CaM-dependent PK II. Two-dimensional gel analysis revealed that the $\alpha$-subunit of $Ca^{2+}$/CaM-dependent PK II is virtually the only synaptic vesicle protein that seems to be labelled after crosslinking (Fig. 2*a*). Chemical modification did not significantly alter the binding constants of the C-terminal fragment of synapsin I for synaptic vesicles (not shown). Consistent with these findings, a 10–20-fold excess of unlabelled synapsin I or exposure to high ionic strength blocked the transfer of $^{125}I$ to the $\alpha$-subunit of $Ca^{2+}$/CaM-dependent PK II by inhibiting the binding of the labelled fragment of synapsin I to synaptic vesicles[7] (Fig. 2*b*). When associated with two other rat brain subcellular fractions that are not enriched in synaptic vesicles, $Ca^{2+}$/CaM-dependent PK II was poorly labelled or not labelled at all after crosslinking (Fig. 2*c*), indicating that not all of the particulate forms of $Ca^{2+}$/CaM-dependent PK II are equivalent with respect to the interaction with synapsin I. The reason for the selective labelling of the synaptic vesicle-associated $\alpha$ subunit is unknown. Distinct higher-order structural features of the vesicle-associated kinase subunits, as well as interactions of synapsin I with phospholipids and/or other vesicle-specific proteins, may contribute to the selectivity of the interaction of $Ca^{2+}$/CaM-dependent PK II with synapsin I.

Synaptic vesicle-associated $Ca^{2+}$/CaM-dependent PK II was catalytically active in phosphorylating various vesicle proteins on addition of $Ca^{2+}$/calmodulin (Fig. 3*a*). The main phosphorylated proteins were identified by immunoprecipitation and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as synapsin I (Fig. 3*b*) and the $\alpha$- and $\beta/\beta'$ subunits of $Ca^{2+}$/CaM-dependent PK II (not shown). The addition of purified soluble $Ca^{2+}$/CaM-dependent PK II to the assay mixture did not significantly increase the extent of synapsin I phosphorylation (Fig. 3*a*). The phosphorylation reaction was specific for $Ca^{2+}$/CaM-dependent PK II, as it was inhibited by CKIP-1 (Fig. 3*a*, *b*), a selective peptide inhibitor of $Ca^{2+}$/CaM-dependent PK II, with an apparent 50% inhibitory concentration ($IC_{50}$) of 10 $\mu$M, but not by selective peptide inhibitors of cyclic AMP-dependent protein kinase or protein kinase C (not shown).

The unlabelled and purified C-terminal fragment of synapsin I bound to synapsin I-depleted vesicles under conditions in which $Ca^{2+}$/CaM-dependent PK II was inactive (Fig. 3*c*). The addition of $Ca^{2+}$/calmodulin in the presence of $Mg^{2+}$/ATP led to phosphorylation of the C-terminal fragment of synapsin I and to its dissociation from the vesicle membrane. The stoichiometry of phosphorylation of the dissociated synapsin I C-terminal fragment (1.5–1.8 mol phosphate per mol protein) was higher than that of the bound fragment (0.8–1.0 mol phosphate per mol protein). The addition of purified exogenous $Ca^{2+}$/CaM-dependent PK II did not significantly increase either the phosphorylation or the extent of dissociation of the synapsin I C-terminal fragment. Autophosphorylation of the vesicle-associated $Ca^{2+}$/CaM-dependent PK II did not promote its translocation to the supernatant. In contrast, the exogenously added $Ca^{2+}$/CaM-dependent PK II was consistently found in the soluble fraction.

To identify the domain(s) of $Ca^{2+}$/CaM-dependent PK II involved in the interaction with the synapsin I C-terminal region, we tested whether the binding of the C-terminal fragment of synapsin I was inhibited by peptides corresponding to sequences of the autoregulatory domain (CKIP-1 and CKIP-2 peptides) or association domain (A1 and A2 peptides) of the kinase or by peptides containing the $Ca^{2+}$/CaM-dependent PK II phosphorylation sites of synapsin I (S2 and S3 peptides) (Fig. 4). The CKIP-1 and CKIP-2 peptides inhibited the binding of the C-terminal fragment of synapsin I to synapsin I-depleted synaptic vesicles, with $IC_{50}$ values of 8.2 and 3.7 $\mu$M, respectively (Fig. 4*a*). Parallel inhibition of $^{125}I$-incorporation into the $\alpha$-subunit of $Ca^{2+}$/CaM-dependent PK II by the two peptides was observed in cross-linking experiments with the modified C-terminal fragment of synapsin I (not shown). The inhibitory effect of these peptides can be ascribed to a direct interaction

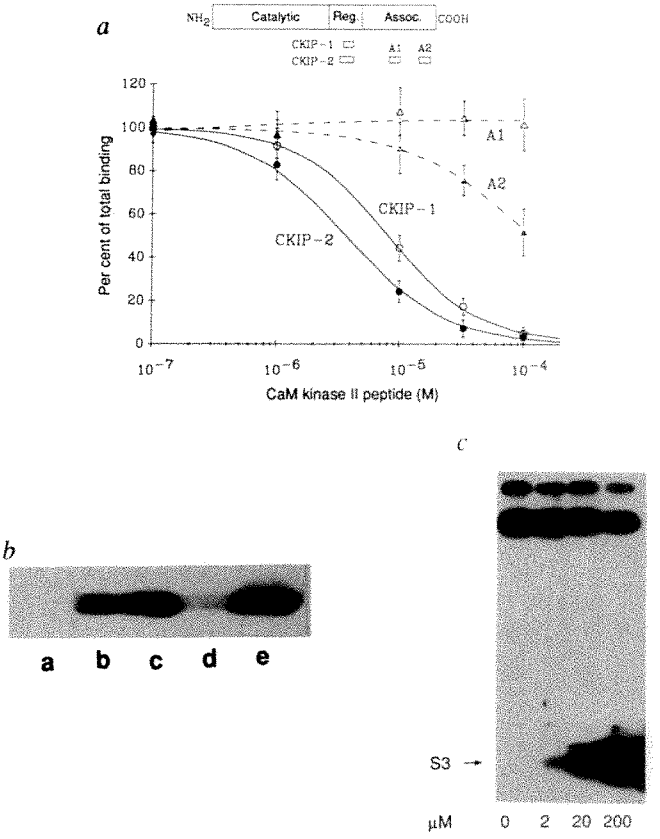



FIG. 4 Binding of the C-terminal fragment of synapsin I to the regulatory domain of $Ca^{2+}$/CaM-dependent PK II. *a*, The dephosphorylated C-terminal fragment of synapsin I was bound to synapsin I-depleted synaptic vesicles in the absence or presence of various concentrations of $Ca^{2+}$/CaM-dependent PK II peptides with no $Mg^{2+}$/ATP or $Ca^{2+}$/CaM in the medium. The amount of bound fragment, quantified using immunoblotting with synapsin I-specific antibodies, is expressed as percentage of the amount bound in the absence of peptides. Values are means (±s.e.m.) of five experiments. Inhibition curves were computed using the computer program ALLFIT[26]. The peptides used were CKIP-1, CKIP-2 ($Ca^{2+}$/CaM-dependent PK II $\alpha$-subunit (273–302)Ala[286]), A1 ($Ca^{2+}$/CaM-dependent PK II $\alpha$ subunit (378–402)) and A2 ($Ca^{2+}$/CaM-dependent PK II $\alpha$ subunit (442–467)). *b*, Autoradiography of a region of a 15% SDS-polyacrylamide gel analysing the high-speed supernatant of samples in which the modified, $^{125}I$-labelled C-terminal fragment of synapsin I was incubated with synapsin I-depleted synaptic vesicles in the absence (a) or in the presence of 30 $\mu$m CKIP-2 (b), 100 $\mu$m CKIP-2 (c), 30 $\mu$m CKIP-1 (d) or 100 $\mu$m CKIP-1 (e). All samples were exposed to ultraviolet light (254 nm) for 2 min. The region of the gel depicted in the figure represents the position in which molecular weight standards in the range of 1–3K were located. *c*, Absence of effect of S3 peptide, corresponding to one of the $Ca^{2+}$/CaM-dependent PK II phosphorylation sites of synapsin I (site 3 peptide, bovine synapsin I (587–609)), on the binding of the C-terminal fragment of synapsin I to synapsin I-depleted synaptic vesicles. The binding was assessed in the presence of the indicated concentrations of S3 peptide, with neither $Mg^{2+}$/ATP nor $Ca^{2+}$/CaM in the medium. After high-speed centrifugation, the proteins in the pellet were analysed by SDS-PAGE followed by immunoblotting with an antibody directed against the S3 peptide. The immunoreactive doublet in the upper part of the figure corresponds to the C-terminal fragments of synapsins Ia and Ib bound to synaptic vesicles.

with the C-terminal fragment of synapsin I because, after photolabelling, $^{125}$I-labelled peptides were recovered in the supernatant (Fig. 4*b*). These results indicate an involvement of the regulatory domain of the kinase in the binding of the C-terminal fragment of synapsin I. The A1 and A2 peptides, corresponding to regions within the association domain of the kinase, had little or no effect.

The synapsin I S3 peptide, containing phosphorylation site 3, bound to synaptic vesicles (Fig. 4*c*), presumably to the catalytic domain of $Ca^{2+}$/CaM-dependent PK II. The same peptide (which is an efficient substrate for soluble $Ca^{2+}$/CaM-dependent PK II ($K_m$, 50 μM)) did not inhibit the binding of the C-terminal fragment of synapsin I, indicating that this latter binding does not involve an interaction with the catalytic domain of the kinase. Similar results were obtained with the S2 peptide (rat synapsin I, residues 561–570, containing phosphorylation site 2) or with the combination of S2 and S3 peptides (not shown).

On the basis of its ability to phosphorylate a wide variety of substrates, $Ca^{2+}$/CaM-dependent PK II has been called a multifunctional protein kinase[12–14]. The high levels of $Ca^{2+}$/CaM-dependent PK II[15,16] or of a related molecule[17], observed in subcellular fractions, such as the postsynaptic density, have led to speculation of a possible structural role for this enzyme. We have now shown that a synaptic vesicle-associated form of $Ca^{2+}$/CaM-dependent PK II serves as a binding protein for synapsin I. The interaction of synapsin I with vesicle-associated $Ca^{2+}$/CaM-dependent PK II, and as a consequence its biological function, is modulated by the catalytic activity of the enzyme. Finally, as a preformed complex of synapsin I and $Ca^{2+}$/CaM-dependent PK II on vesicles exists, it is conceivable that, upon localized $Ca^{2+}$ entry at the presynaptic active zone, the rate of phosphorylation of synapsin I by $Ca^{2+}$/CaM-dependent PK II might be sufficiently rapid to play a direct role in the events mediating exocytosis. □

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# Evidence for cooperative interactions in potassium channel gating

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**CLONING and expression of voltage-activated potassium ion-channel complementary DNAs[1–4] has confirmed that these channels are composed of four identical subunits[5], each containing a voltage sensor. It has been generally accepted that the voltage sensors must reach a permissive state through one or more conformational ('gating') transitions before the channel can open[6,7]. To test whether each subunit gates independently, we have constructed cDNAs encoding four subunits on a single polypeptide chain, enabling us to specify the subunit stoichiometry. The gating of heterotetramers made up from combinations of subunits with different gating phenotypes strongly suggests that individual subunits gate cooperatively, rather than independently[8]. Nonindependent subunit gating is consistent with measurements of the kinetics of $K^+$-channel gating currents[9–13] and in line with the widespread subunit cooperativity observed in other multisubunit proteins[14].**

To manipulate the subunit composition of an RCK1 $K^+$-channel[7,15,16], we constructed cDNAs encoding two[17–19] or four[20,21] RCK1 subunits on a single cDNA (Fig. 1). Figure 2*a* shows that $K^+$-channels expressed in *Xenopus* oocytes from cDNAs encoding wild-type (WT) dimers or tetramers have gating phenotypes that are indistinguishable from a WT RCK1 monomer. We have obtained strong evidence that most channels expressed from a cDNA encoding all four subunits are composed of the subunits specified on that cDNA, and that formation of heteromultimeric channels from subunits coming together from different polypeptide chains is negligible. This conclusion is based on the following findings. (1) Although the rate of tail current deactivation in tetramers containing mixtures of WT and mutant subunits can differ substantially from that of WT channels, the time course of deactivation is always monoexponential, implying that a single population of channels is expressed. (2) cDNAs coding for WT-X dimers, where X is a nonexpressing subunit (R3I, K5I, R6N, see ref. 22), give no functional expression, implying that assembly of WT subunits from different polypeptide chains into functional homotetramers does not take place if WT and mutant subunits are present on the same polypeptide chain. The corresponding tetramers WT-X-WT-X do not express functional channels either. (3) Phenotypes for dimers are identical, regardless of the order in which the different subunits are encoded (Fig. 2*b*). (4) All channel cDNAs are cloned into a high-expression vector in which the channel cDNAs are flanked by 5′ and 3′ untranslated regions from a *Xenopus* β-globin gene[23]. This construct enhances expression by a factor of 100–1,000. The 3′-untranslated region by itself enhances expression by a factor of 4–10 (E. Liman, J.T. and P.H., unpublished results), thus favouring the translation of full-length messenger RNAs. (5) Dose–response curves for tetraethylammonium (TEA) in tetramers containing either 1, 2, or 3 TEA-insensitive mutant subunits are each fitted by a single and distinct inhibition constant ($K_i$) (ref. 18, and E. Liman, J.T. and P.H., unpublished results).

To test for interactions between individual subunits in the process of channel activation, we constructed dimeric and tetrameric cDNAs encoding combinations of two kinds of subunits with different gating phenotypes. Figure 3 shows steady-state activation curves for combinations of WT subunits and subunits in which the second arginine residue in S4 has been

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replaced by asparagine (R2N). We chose the R2N mutant because of its very pronounced gating phenotype[22]. Each of the constructs containing a different combination of WT and R2N subunits led to a distinct phenotype. The smooth curves superimposed on the datapoints in Fig. 3*a* are predictions from a model of steady-state activation in which each subunit gates independently. Once parameters are chosen to fit activation curves for all-WT and all-R2N channels separately, no further adjustable parameters are needed to predict the activation curves of channels with combinations of WT and R2N subunits. For each of the WT-R2N combinations shown in Fig. 3*a*, the observed shift of the activation curve is substantially larger than that predicted by our model with independent gating. If, however, subunits were gating in a cooperative way, the presence of an R2N subunit will tend to promote the conformational change of a neighbouring WT subunit, thus shifting the activation curve to more negative potentials. A fit of the same data to a model with positive cooperativity is shown in Fig. 3*b*. Here, the forward (opening) transition rate constant for each subunit is multiplied by a cooperativity factor, $k_{co\text{-}op}$ (=6), and the backward rate constant divided by $k_{co\text{-}op}$ (=6), if a neighbouring subunit has already undergone the voltage-dependent forward transition. With an equilibrium cooperativity factor of 36, the observed activation curves for all WT-R2N combinations are predicted accurately.

Figure 4 shows activation curves obtained with tetramers made of combinations of WT subunits and subunits in which the fourth arginine residue in S4 had been mutated to isoleucine (R4I; ref. 22). With each substitution of a WT subunit by an R4I mutant, the activation curve is shifted towards more negative potentials and the steepness of activation decreases (Fig. 4*a*). A plot of the limiting slope of activation for each WT-R4I

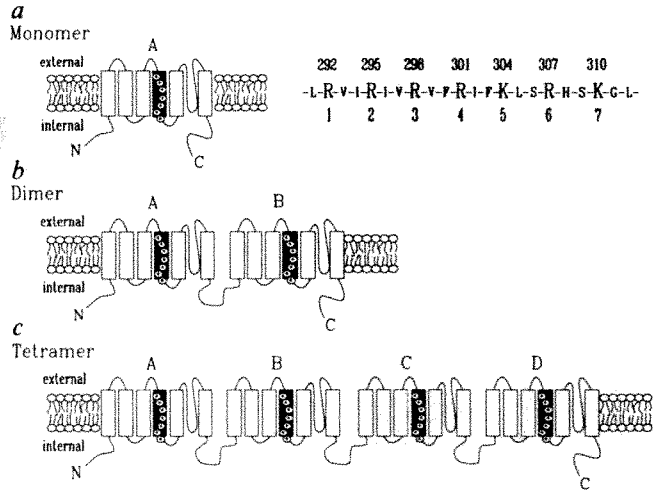



FIG. 1 Construction of cDNAs. *a*, Putative transmembrane folding scheme for a monomeric subunit of voltage-dependent $K^+$-channels (left) with the S4 sequence for RCK1 (right). The complete sequence of RCK1 can be found in refs 15 and 16. *b*, *c*, Scheme for dimeric and tetrameric constructs.
METHODS. All cDNAs were cloned into a 3 kilobase (kb) high-expression vector based on pGEM3Z (Promega) containing 5′ and 3′ nontranslated sequences of a *Xenopus* β-globin gene[23] flanking the channel cDNA. For *in vitro* transcription, the plasmid was linearized 3′ to the 3′ nontranslated β-globin sequence. Dimeric cDNA: monomeric RCK1 cDNA was flanked by an *Eco*RI (5′) and a *Hin*dIII site (3′). For the construction of dimeric cDNA, the *Eco*RI site was mutated to a *Bam*HI site and a new *Eco*RI site was created before the stop codon, 5′ to the *Hin*dIII site. After digestion of this cDNA (channel A) and of an original RCK1 channel (channel B) with *Eco*RI (5′) and *Hin*dIII (3′), channel B could be ligated in-frame after channel A, creating a dimeric cDNA. The link between the two channels thus contained the entire C terminus of channel A, the entire N terminus of channel B, plus 17 new amino acids derived from the 5′ nontranslated region of the RCK1 clone of channel B between the *Eco*RI site and the normal initiation codon. The sequence of the 17 amino acids (single-letter code) is: LHPGLSPGLLPLHPASI. Tetrameric cDNA: dimeric cDNA was cut with *Kpn*I (single site at 5′ end of nontranslated β-globin sequence) and *Dra*I (single site after first base of stop codon in channel B), yielding a 3-kb fragment containing channels A and B. A plasmid containing channel C was cut with *Xma*I (single site at bases coding for first PG amino acids of link listed above), blunted with Klenow fragment of DNA polymerase and recut with *Kpn*I, yielding a 4.5-kb fragment containing the vector and channel C. Ligation of the two fragments (A, B+C) produced trimeric cDNA (channels A, B, C), with a single remaining *Dra*I site at the end of channel C. In an iterative way channels A, B, C were then excised as a 4.5-kb fragment with *Kpn*I and *Dra*I and ligated to a 4.5-kb fragment (*Kpn*I to blunted *Xma*I) containing the vector and channel D to yield tetrameric cDNA (channels A, B, C, D). The links between the C and N termini of channels B and C, and C and D, contained the last 15 of the 17 amino acids listed above. For site-directed mutagenesis the Altered Sites system (Promega) was used. Mutations were verified by dideoxy DNA sequencing. For each construct, several independent clones were sequenced, transcribed and expressed.

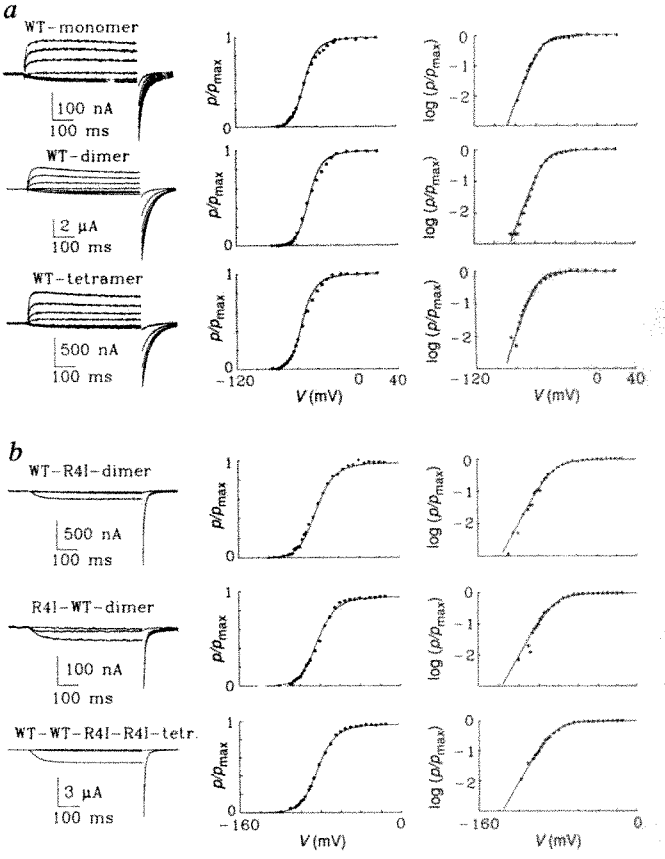
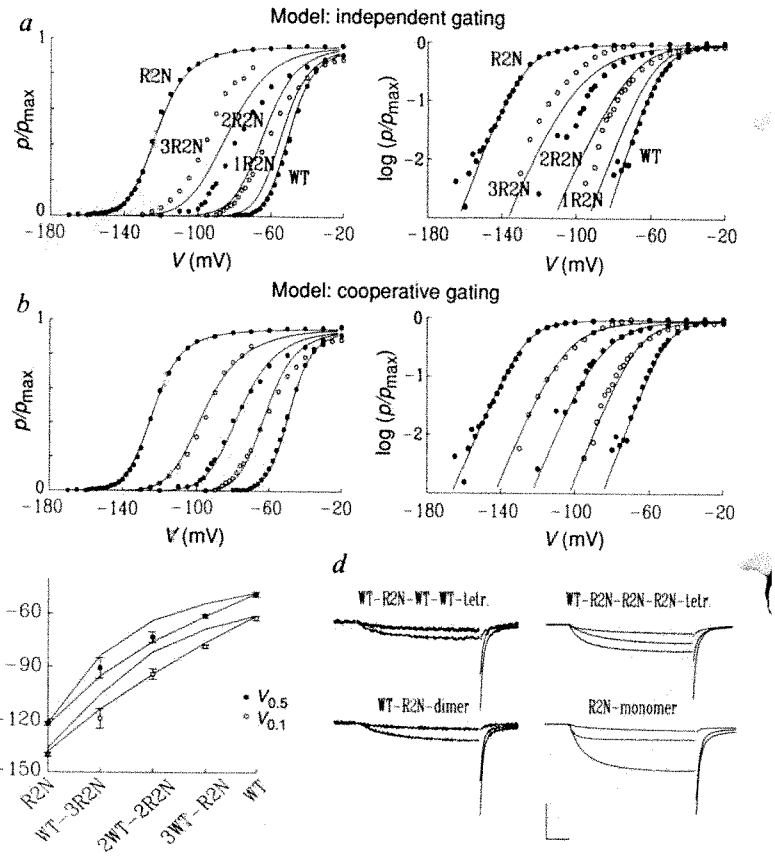



FIG. 2 Channels encoded by monomeric, dimeric or tetrameric RCK1 cDNAs with current records (left column) and steady-state activation curves (middle and right) from oocytes injected with RNA *in vitro* transcribed. *a*, WT monomeric, WT dimeric and WT tetrameric cDNA. Holding potentials, −100 mV (monomer) and −110 mV (dimer and tetramer). Test potentials for WT currents shown ranged from −60 to 0 mV in 10 mV increments. Steady-state activation curves were obtained as relative amplitudes of the tail currents elicited by repolarization from the test potentials indicated on the *x*-axis. Mean voltages (±s.e.m.) for 10% and 50% activation: −65±1 and −53±1 mV (monomer, $n=10$), −65.1±2 and −52.5±2 mV (dimer, $n=5$), −69.5±3.1 and −56.6±2.4 mV (tetramer, $n=5$). Mean equivalent gating valences $z$, respectively 6.7±0.2, 6.8±0.2 and 6.7±0.5. *b*, WT-R4I dimeric, R4I-WT dimeric and WT-WT-R4I-R4I tetrameric cDNA. Holding potential, −140 mV. Test potentials were −110, −60 and −40 mV. Mean voltages for 10% and 50% activation, −102.4±2 and −82±2 mV (WT-R4I dimer, $n=42$), −99.3±1.9 and −78.7±2.3 mV (R4I-WT dimer, $n=14$), −100.4±1.4 and −81.3±2 mV (WT-WT-R4I-R4I tetramer, $n=12$). Mean $z$ values, respectively 4.2±0.3, 4.3±0.3 and 4.7±0.4. Two-microelectrode voltage-clamp recordings were as previously described[7,22]. Bath solution (in mM): 50 RbCl, 70 NaCl, 0.3 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.2; $p$ is the open probability, and $p_{max}$, the maximal open probability; $p/p_{max}$ represents the fractional activation.

FIG. 3 Steady-state activation curves for channels consisting of WT and mutant subunits suggest cooperative gating. *a, b*, Steady-state activation curves for WT and R2N channels, WT-R2N dimers (2R2N), or tetrameric combinations in which either 1 (1R2N, R2N in position B), or 3 (3R2N, WT in position A) WT subunits were substituted by an R2N subunit. Experimental conditions as in Fig. 2. Solid curves are model predictions for independent (*a*) or cooperative gating (*b*). Model: Each subunit can assume a nonpermissive and a permissive state, with voltage-dependent rate constants for the forward ($k_f$) and backward ($k_b$) transitions. $k_f = k_{f0} \cdot \exp(z_f VF/RT)$ and $k_b = k_{b0} \cdot \exp(-z_b VF/RT)$, where $k_{f0} = k_f$ at $V = 0$, $k_{b0} = k_b$ at $V = 0$, $z_f$ and $z_b$ are the valences of the forward and backward gating transitions, and $F$ is the Faraday constant. The model has 16 closed states, corresponding to all possible permutations of four subunits. The closed state with all subunits in the permissive conformation is linked to a single open state by voltage-independent rate constants (opening rate constant $k_o = 30{,}000\ s^{-1}$, closing rate constant $k_c = 2{,}200\ s^{-1}$; see refs 7, 22). Parameters for independent model: WT, $k_{f0} = 2{,}447\ s^{-1}$, $k_{b0} = 94\ s^{-1}$, $z_t = z_f + z_b = 1.7$ electronic charges ($e$) per sensor; R2N, $k_{f0} = 18{,}100\ s^{-1}$, $k_{b0} = 12\ s^{-1}$, $z_t = 1.5\ e$. Cooperative model: WT, $k_{f0} = 376\ s^{-1}$, $k_{b0} = 645\ s^{-1}$, $z_t = 1.3\ e$; R2N, $k_{f0} = 1{,}507\ s^{-1}$, $k_{b0} = 153\ s^{-1}$, $z_t = 1.1\ e$. In the cooperative model, a factor $k_{co\text{-}op}$ was defined such that for the transition of a subunit with a neighbouring subunit in the permissive state, $k_f$ was set to $k_f \cdot k_{co\text{-}op}$ and $k_b$ to $k_b/k_{co\text{-}op}$. The curves in *b* were obtained with $k_{co\text{-}op} = 6$. *c*, Voltages of 50% activation ($V_{0.5}$, filled symbols) and 10% activation ($V_{0.1}$, open symbols) for the indicated channel constructs. Symbols are mean ±s.e.m. of 6, 4, 6, 4 and 35 measurements (left to right). Curves are model fits with the same parameters as in *a* and *b*. The more positive voltages are those predicted by the independent model. *d*, Current traces, WT-R2N-WT-WT with holding potential (h.p.) −140 mV and test potentials (t.p.) −80, −60 and −40 mV, WT-R2N (h.p. −160 mV, t.p. −100, −70 and −40 mV), WT-R2N-R2N-R2N (h.p. −160 mV, t.p. −120, −100 and −80 mV) and R2N (h.p. −180 mV, t.p. −140, −100 and −60 mV). Scale bars, 100 ms and 200 nA (left traces), 2 μA (right).



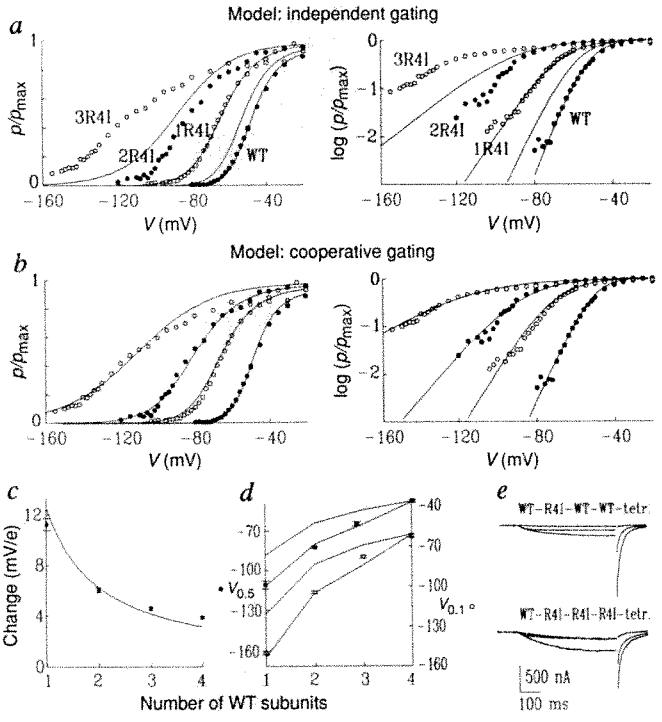




FIG. 4 Tetramers containing combinations of WT and R4I mutant subunits. *a, b*, Steady-state activation curves from WT channels and tetrameric cDNAs in which either 1 (1R4I), 2 (2R4I) or 3 (3R4I) WT subunits were substituted with mutant subunits R4I. Solid lines, model predictions for independent (*a*) or cooperative gating (*b*). Model parameters for WT subunits as in Fig. 3. For R4I subunits, $k_{b0} = 0$, that is, the activation of R4I subunits is assumed to be shifted to infinitely negative potentials. In *b*, $k_{co\text{-}op} = 6$, as in Fig. 3*b*. *c*, Exponential slopes of steady-state activation curves at low values of $p$, expressed as change in terms of mV/$e$ of fractional activation. Values are means ±s.e.m. from 24, 70, 9 and 35 oocytes (left to right). The solid line indicates the expected slopes for the case in which the observed voltage dependence of activation was contributed by the WT subunits. *d*, Voltages of 50% activation ($V_{0.5}$, filled symbols) and of 10% activation ($V_{0.1}$, open symbols) for WT-R4I combinations. Symbols are means ±s.e.m. of 24, 70, 9 and 35 measurements (left to right). Curves are model fits with same parameters as in *a* and *b*. The more positive voltages are those predicted by the independent model. *e*, Current traces: WT-R4I-WT-WT (h.p. −120 mV, t.p. −90, −70 and −40 mV), WT-R4I-R4I-R4I (h.p. −180 mV, t.p. −150, −120 and −80 mV).

combination shows that the voltage-dependence of activation is nearly inversely proportional to the number of WT subunits (Fig. 4*c*). The data thus imply that R4I subunits are stuck in the 'permissive' conformation. Messenger RNA coding for R4I subunits alone failed to produce measurable time-dependent currents in oocytes, consistent with a permanent inactivation at holding potentials that are much more positive than the voltage range of activation[24].

Tetramers of WT–R4I combinations give us another way to test for cooperative gating. Figure 4*a* shows the maximal shifts in activation curves that the model with independent subunit gating can produce if it is assumed that R4I channels never

close. As in the case of the WT-R2N tetramers, the observed voltage shifts considerably exceed the ones predicted by the noncooperative model. In contrast, the cooperative model, with the same $k_{co\text{-}op}$ (=6) and equilibrium cooperativity factor (=36) used to fit the WT-R2N combinations, nicely fits the activation curves of the WT-R4I mixtures (Fig. 4*b*, *d*).

Our conclusions about cooperative gating of individual voltage sensors are derived from model fits to steady-state activation curves. We do not wish to imply that our model with a single voltage-dependent gating transition for each subunit is mechanistically correct, as other gating schemes with more transitions can equally well be made to fit a particular activation curve. But cooperativity among subunits seems to provide a simple explanation for the observed gating phenotype[9-13,25].

We also show that by constructing cDNAs encoding tetrameric $K^+$-channels, we can reproducibly manipulate the subunit composition. Finally, WT-R4I tetramers illustrate that changes in the steepness of activation cannot be used to estimate the total equivalent gating charge of a channel in which a mutation confined to a single domain or subunit has caused a voltage shift of that voltage sensor[26]. □

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# Transcriptional activation by the human c-Myc oncoprotein in yeast requires interaction with Max

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**THE c-*myc* protein (Myc) contains an amino-terminal transcriptional activation domain[1] and a carboxy-terminal basic helix–loop–helix–leucine zipper (bHLH-Z) domain[2-5] that directs dimerization of Myc with its partner, the *max* protein (Max), and promotes DNA binding to sites containing a CACGTG core consensus sequence[6-9]. Despite these characteristics and the observation that Myc can modulate gene expression[4,5,10], a direct role for Myc or Max as transcription factors has never been demonstrated. Here we use *Saccharomyces cerevisiae* as an *in vivo* model system to show that the Myc protein is a sequence-specific transcriptional activator whose DNA binding is strictly dependent on dimerization with Max. Transactivation is mediated by the amino-terminal domain of Myc. We find that Max homodimers bind to the same DNA sequence as Myc+Max but that they fail to transactivate and thus can antagonize Myc+Max function. We also show that the Max HLH-Z domain has a higher affinity for the Myc HLH-Z domain than for itself, and suggest that the heterodimeric Myc+Max activator forms preferentially at equilibrium.**

Analysis of transcriptional regulation by Myc and Max proteins in mammalian cells is limited by endogenous Myc and Max. As there is no evidence for Myc and Max homologues in *S. cerevisiae*, this organism seems ideal for such a study. Co-expression of human Myc with Max1, Max2 or one of several

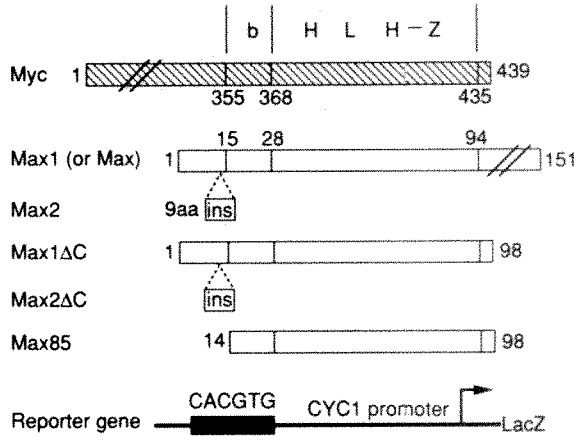


FIG. 1 Schematic representation of human c-Myc, Max, and Max deletion derivatives, and of the CACGTG-CYC1-LacZ reporter gene. The bHLH-Z domains of Myc and Max are aligned. Max1 and Max2 are the natural Max variants without and with the 9-amino acid (9aa) insert, respectively[7]. The MaxΔC and Max85 mutants are truncated at the position in Max equivalent to the Myc carboxy-terminus.

METHODS. Max2, Max1ΔC, Max2ΔC, and Max85 were generated by polymerase chain reaction (PCR) with appropriate primers from a Max1 complementary DNA template (a gift from D. Gillespie). All the coding regions were subcloned into galactose-inducible CEN-ARS plasmids of the pSD series of yeast expression vectors[15], with either TRP1, LEU2 or HIS3 as selective markers. Because we used the CYC1 ATG initiation codon, the following N-terminal extensions precede the proteins: MTGFPGLQEFELAPTM (Myc), MTGFELE (Max1 and Max103-VP16; Fig. 2), MTGFT (Max2, Max1ΔC, Max2ΔC), and MTGFTMG (Max85), and MTGFPG (MycΔN; Fig. 2) (single-letter amino-acid code). Additional experiments with proteins initiated at their own start codons indicated that the extensions do not significantly alter the behaviour of Myc, Max1, Max2, Max1ΔC and Max2ΔC (data not shown). All chimaeric fusions and inserts generated by PCR were verified by DNA sequencing. As a reporter gene we used a 2μ-CYC1 promoter-LacZ plasmid derived from pLGΔ312 (ref. 28) containing the adenovirus major late promoter element GTAGGCCACGTGACCGGGTGT (ref. 29) inserted between the *Sma*I and *Xho*I sites. Two other Myc binding sequences (CM1; ref. 6 and the PHO4 promoter[13]) gave similar results.

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Max deletion derivatives (Fig. 1) in yeast cells leads to transcriptional activation from a CACGTG-CYC1-LacZ reporter, whereas no activation is observed with either Myc or Max alone (Fig. 2*a*, lanes b). To assess whether the failure of Myc or Max alone to transactivate was due to an inability to bind DNA, we tested their function when fused to a heterologous transactivation domain (VP16; Fig. 2*b*). As seen with Myc, VP16-MycΔN (retaining Myc residues 180–439) transactivates only when co-expressed with Max. This is consistent with the idea that DNA binding and therefore transactivation by Myc requires association with Max (either Max1 or Max2) or at least the Max bHLH-Z domain (Max85; Figs 1 and 2*a*). Unlike VP16-MycΔN, Max103-VP16 by itself transactivates the CACGTG-CYC1 promoter (Fig. 2*b*). Thus, Max can bind DNA in the absence of Myc but does not significantly activate transcription in our system unless tagged with a heterologous transactivation domain. Accordingly, all Max proteins tested (Fig. 1) bind DNA as homo-oligomers in yeast extracts (ref. 9, and data not shown), as recently reported for bacterially expressed Max[11,12]. Transactivation of the CACGTG-CYC1 promoter is sequence-specific, as much less or no transactivation by Myc + Max (Fig. 2*a*, lanes a), VP16-MycΔN + Max or Max103-VP16 (Fig. 2*b*) is observed from a control reporter lacking the CACGTG binding site (see also Fig. 2*a* legend). Although yeast bHLH proteins that bind to the same DNA sequence as Myc and Max have been described[13], they are unlikely to play a significant role in our experiments.

Because of their apparent lack of transcriptional activity, we expected Max + Max dimers to antagonize transactivation by Myc + Max through competition for the same DNA target site. Consistent with this idea, introducing an additional Max plasmid into the cells leads to a reduction of transactivation by Myc + Max or Myc + Max85 (Fig. 2*c*). Introduction of an additional Myc plasmid enhances transactivation levels in the presence of Max or Max85 (Fig. 2*c*), supporting the conclusion that Myc provides the activation domain. Thus, the activities of different Myc + Max dimers primarily reflect the equilibrium between Myc + Max and Max + Max complexes rather than their absolute efficiencies (Fig. 2). For example, MaxΔC and Max85 proteins seem to allow better Myc + Max function than full-length Max (Fig. 2*a*), although all Max proteins were expressed at similar levels (data not shown). Both truncated proteins lack the C-terminal nuclear import sequence[11,14] and may therefore be less effective as homodimeric competitors, whereas the respective heterodimers with Myc are efficiently transported into the nucleus[14].

Given that Max can interact both with itself and with Myc, we investigated the relative affinities of the Myc and Max HLH-Z domains for themselves and for each other using a yeast assay that monitors protein–protein interactions *in vivo*[15,16] (Fig. 3*a*). We observed efficient Myc–Max HLH-Z interaction but no Myc–Myc and weak Max–Max HLH-Z interactions (Fig. 3*b*). Our results in yeast are similar to observations from a similar assay in mammalian cells[11], although in that case no Max–Max interactions were detected. The relative dimerization affinities of Myc and Max HLH-Z domains are reminiscent of the inter-

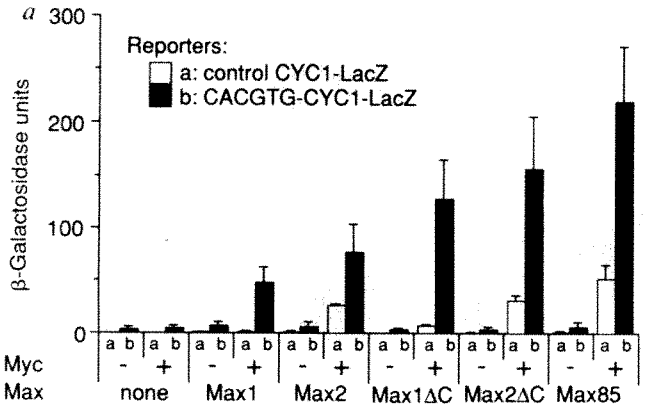
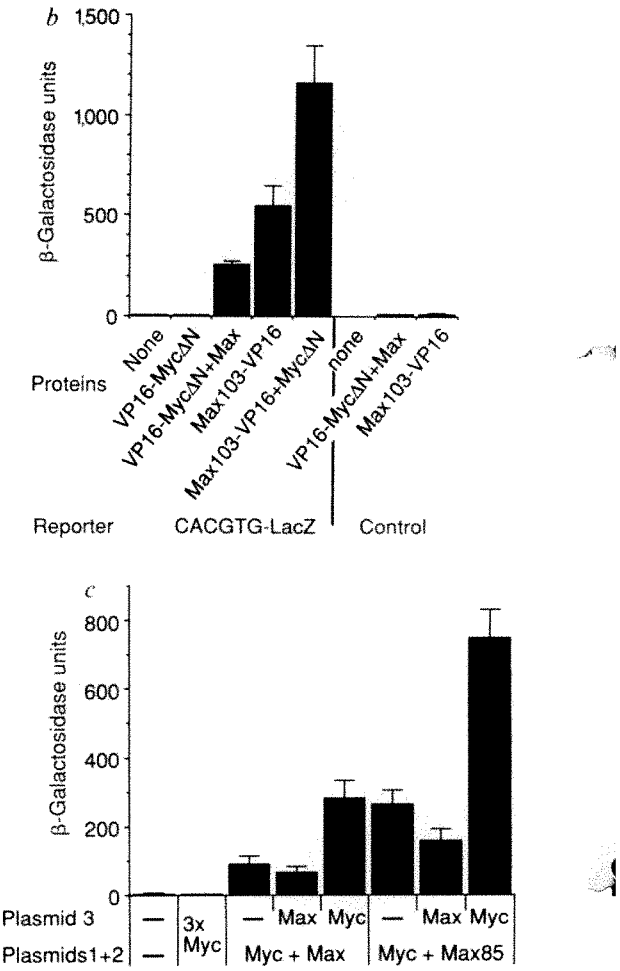
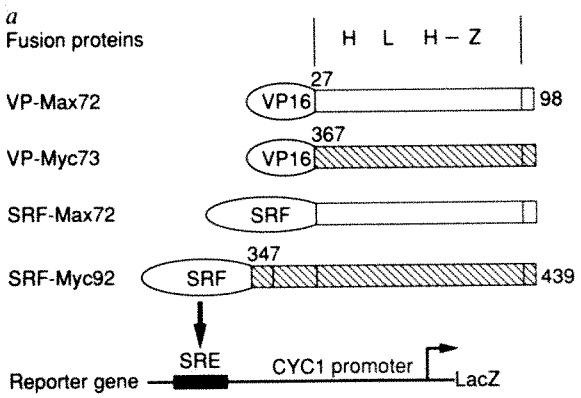
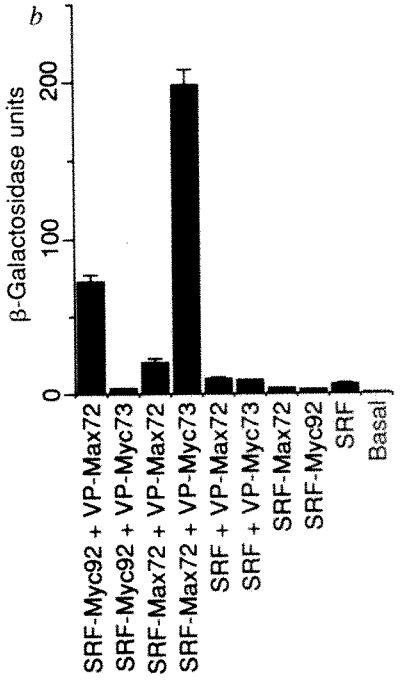



FIG. 2 Transcriptional activation by Myc and Max in yeast. *a*, β-Galactosidase units in cells containing the indicated proteins and reporters. Residual transactivation of the control reporter may be due to two CACATG boxes in the CYC1 promoter[30]. CACATG is the only half-site change permitting DNA binding by Myc + Max, Max1 or Max2, albeit with decreased affinity[11] (D. Solomon, B.A. and H.L., unpublished data). All Max proteins tested were expressed at similar levels, whereas Myc levels were too low to be detected by immunoblotting (not shown). The relatively higher efficiencies of Myc with Max2, Max2ΔC or Max85, compared with Max1 or Max1ΔC, may in part be due to differences in affinity for DNA (ref. 9, and data not shown). *b*, Transactivation by VP16-MycΔN and Max103-VP16 together with Max or MycΔN. VP16-MycΔN and Max103-VP16 activities cannot be directly compared as their relative expression levels are unknown and VP16-MycΔN + Max is antagonized by Max + Max in cells (see text). *c*, Effect of a third Myc or Max plasmid on transactivation of the CACGTG reporter. Myc plasmids with different genetic markers were used for Myc + Max and Myc + Max85 (LEU2 in *a*, HIS3 in *c*).

METHODS. Max103-VP16 was constructed by replacing Max sequences 3′ of codon 103 with a VP16 fragment (residues 410–490) from pSD.06a (ref. 15). VP16-MycΔN contains the VP16 fragment upstream of Myc codons 180 to 439. MycΔN retains codons 178 to 439. Myc and Max plasmids were transformed into the yeast strain W303-1B (MAT$\alpha$ *ho ura3 his3 trp1 ade2 leu2 can1-100*) and reporter plasmids into the isogenic strain W303-1A (MATa). Protein/reporter combinations were generated by crossing transformants. Relative $\beta$-galactosidase units (U) in cultures induced for 12 h with galactose were measured as described[31] and normalized to cell numbers as $U = 1{,}000A_{420}(CVt)^{-1}$, where $A_{420}$ is the absorbance at 420 nm, $C$ is the density of the cell suspension (in $A_{600}$ ml$^{-1}$), $V$ is the volume of cell suspension (ml), and $t$ is the total incubation time (min).





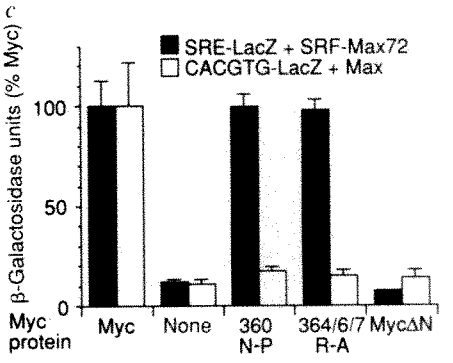




FIG. 3 Myc–Max interactions *in vivo*. *a*, Assay for interactions between Myc and Max HLH-Z domains. In this assay[15], activation of the serum-response element (SRE)–LacZ reporter gene is strictly dependent on the interaction between chimaeric proteins containing the serum-response factor (SRF) DNA-binding and the VP16 transactivation domains, respectively. Schematic representations of the fusion proteins and reporter gene that were used. The SRE–CYC1–LacZ reporter gene is integrated into the genome of the indicator yeast strain S62L (ref. 15). *b*, Relative β-galactosidase units (as defined in legend to Fig. 2) in S62L cells expressing the indicated proteins. The reciprocal combinations used provide an internal functional control for expression of the fusion proteins. Furthermore, all the SRF derivatives used here recruit equally well a SAP1–VP16 fusion protein that binds to the SRF moiety[15] (not shown). *c*, Wild-type Myc and the indicated mutant derivatives were assayed for transactivation of SRE–CYC1–LacZ through dimerization with SRF–Max72 and of CACGTG–CYC1–LacZ through dimerization and DNA binding with max. The activity of each Myc protein in both assays is normalized to wild-type Myc activity (100%). Wild-type Myc does not interact with SRF alone (not shown).

METHODS. Myc92-, Myc73- and Max72-encoding DNA fragments were generated by PCR and subcloned in frame downstream of VP16 or SRF412 (residues 1–412 of SRF) in vectors pSD.06a and pSD.08, respectively[15]. SRF412 alone was expressed from a modified version of pSD.08. Myc point mutants were generated by site-directed mutagenesis using standard methods. The reading frames of all fusions, the point mutations and all the PCR-generated inserts were verified by DNA sequencing.

actions between Jun and Fos leucine zippers (reviewed in ref. 17). We suggest that, as for Jun and Fos[18,19], Myc and Max preferentially form heterodimeric complexes at equilibrium.

The functional domains of Myc were analysed by measuring the dimerization of various Myc mutants with SRF–Max72 (see Fig. 3*a*), and their DNA-binding properties with Max (Fig. 3*c*).

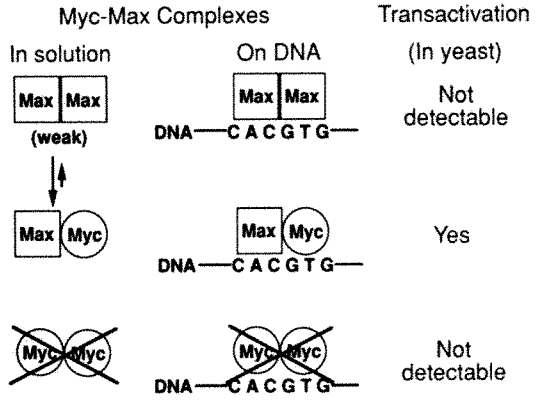



FIG. 4 Schematic summary of Myc and Max interaction and function. Myc and Max form stable heterodimers in the absence of DNA (in solution). Weak Max + Max interactions can also be detected, but Myc + Myc homodimers do not form at physiological concentrations *in vitro* or *in vivo*. Both Myc + Max and Max + Max bind to the same DNA sequence. However, only the Myc + Max heterodimer detectably functions as a sequence-specific transcriptional activator in yeast.

Mutants with substitutions in the basic region (Asn → Pro at residue 360, and Arg → Ala at residues 364, 366 and 367) are unable to transactivate the CACGTG reporter but retain dimerization activity. Thus, as in other bHLH proteins[20,21], the basic region of Myc is essential for DNA binding but dispensable for dimerization, which is mediated by the HLH-Z domain alone (Fig. 3*b*). Consistent with this idea, Myc mutants with deletions of either helix–loop–helix or leucine zipper domains did not show any activity, though positive controls are not available for these mutants (data not shown). The transactivation domain of Myc maps to the 177 N-terminal residues, consistent with the mapping of this domain in GAL4–Myc chimaeras in mammalian cells[1]. The deletion mutant MycΔN (retaining residues 178 to 439) fails to activate in dimerization and in DNA-binding assays (Fig 3*c*), but efficiently enhances transactivation by Max–VP16 (Fig. 2*b*). This shows that MycΔN can dimerize with Max–VP16 and bind DNA but does not transactivate. The Myc transactivation domain also functions in yeast when fused to heterologous DNA-binding domains (LexA–Myc (ref. 22) and 1–235Myc–SRF, data not shown).

Our observations are summarized in Fig. 4. In yeast, DNA binding and therefore transactivation by Myc are dependent on dimerization with Max. As both the transactivation and bHLH-Z domains are essential for Myc function[23–25] (Fig. 3*c*) and Myc and Max are expected to interact in mammalian cells[9,11,26], our results further suggest that the growth-regulatory and oncogenic activities of Myc may depend on gene activation by Myc + Max dimers. Max homo-oligomers bind to the same DNA sequence yet they do not detectably transactivate, and they can antagonize the function of Myc + Max by occlusion of DNA binding sites.

These findings are consistent with the lack of transactivation by GAL4-Max fusions in mammalian cells[11], although a transactivation function for Max cannot be entirely eliminated. Unlike Myc, Max is a stable protein that is expressed in resting as well as in growing cells[26] (T.D.L., D. Hancock and G.I.E., unpublished results). As Myc and Max should preferentially form heterodimers (Fig. 3*b*), we suggest that mitogenic induction of Myc expression[27] leads to a shift in the equilibrium from Max + Max to Myc + Max. This transition may be an important growth-regulatory step, as Myc activity is sufficient to commit cells to the cell cycle[10] or the apoptotic pathway[25], although Myc and Max may also be regulated by phosphorylation[5,12,26].

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# Myc and Max proteins possess distinct transcriptional activities

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**THE Myc family proteins are thought to be involved in transcription[1,2] because they have both a carboxy-terminal basic–helix–loop–helix–zipper (bHLH-Z) domain, common to a large class of transcription factors[3], and an amino-terminal fragment which, for c-Myc, has transactivating function when assayed in chimaeric constructs[4]. In addition, c-, N- and L-Myc proteins heterodimerize, *in vitro* and *in vivo*, with the bHLH-Z protein Max[5–8]. *In vitro*, Max homodimerizes but preferentially associates with Myc, which homodimerizes poorly[5,6]. Furthermore Myc–Max heterodimers specifically bind the nucleotide sequence CACGTG[9] with higher affinity than either homodimer alone[5]. The identification of Max and the specific DNA-binding activities of Myc and Max provides an opportunity for directly testing the transcriptional activities of these proteins in mammalian cells. We report here that Myc overexpression activates, whereas Max overexpression represses, transcription of a reporter gene. Max-induced repression is relieved by overexpression of c-Myc. Repression requires the DNA-binding domain of Max, whereas relief of repression requires the dimerization and transcriptional activation activities of Myc. Both effects require Myc–Max-binding sites in the reporter gene.**

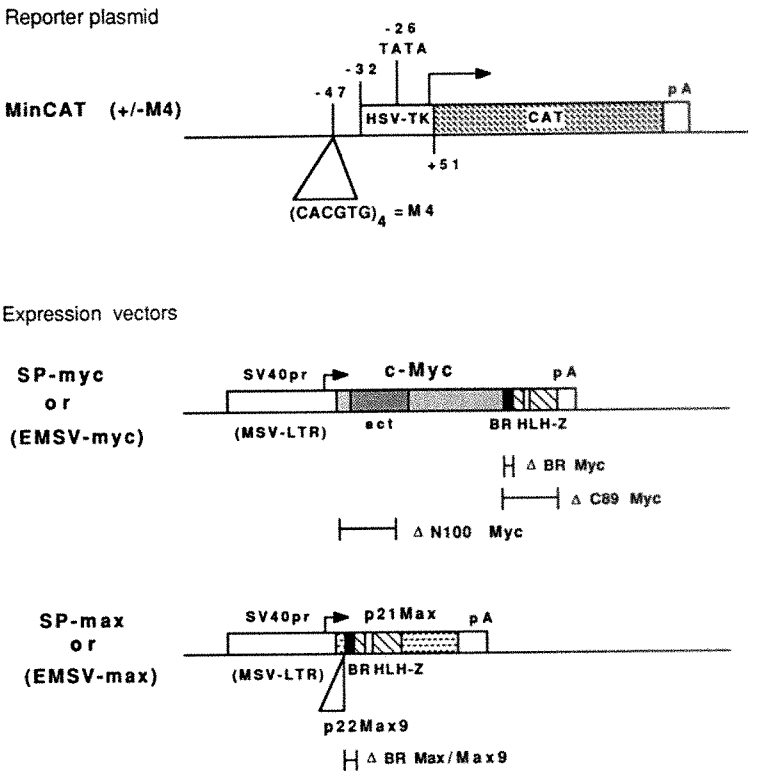
Because no naturally occurring promoter has yet been demonstrated to be a target for Myc or Max regulation we used a chloramphenicolacetyl transferase (CAT) reporter plasmid (MinCAT) with or without a fourfold repeat of the CACGTG Myc/Max-binding sequence (+ or −M4) proximal to a minimal promoter (see Fig. 1*a*). The MinCAT reporter was transfected into fibroblast or epithelial cell lines along with vectors expressing human c-Myc and/or Max proteins (Fig. 1*a*).

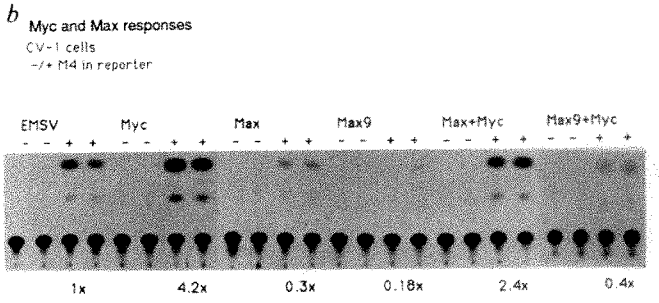
Figure 1*b* demonstrates that, after normalization for transfection efficiency, moderate levels of CAT activity can be detected in CV-1 cells contransfected with M4MinCAT and control expression vector (derived from pEMSV). This endogenous, M4-dependent, activity is expected because proliferating cells express Myc, Max and other CACGTG-binding proteins[7,10–12]. Introducing a vector that overexpresses c-Myc generates a variable increase in CAT activity averaging 3–4-fold over the endogenous level (Fig. 1*b*, *c*), which is comparable to the activation observed *in vivo* for Fos and Jun[13,14]. The degree of activation depends on the concentration of c-Myc expression vector (Fig. 2*c*). Because Myc homodimerizes poorly, overexpressed Myc presumably functions through endogenous Max, which is normally in excess over Myc (E.M.B., unpublished data).

In contrast to the modest activating effect of c-Myc, overexpression of Max or the alternatively spliced Max9 (Fig. 1*a*, and ref. 5) results in significant repression of reporter gene activity (Fig. 1*b*, *c*). Figure 2*a* shows that the extent of repression of endogenous activity depends on the concentration of Max9 expression vector introduced into the cells. To determine whether Max DNA-binding activity is required for the repressive effect, we prepared mutant Max proteins lacking the basic region (ΔBR-Max, Fig. 1*a*). These proteins fail to bind CACGTG oligonucleotides and also inhibit wild-type Max and Myc DNA-binding (unpublished data). Introduction of ΔBR-Max9 into cells failed to inhibit CAT activity from the reporter even at high vector concentrations (Fig. 2*b*), indicating that Max repression requires interaction with the binding site.

The repressive effect of Max overexpression on the reporter gene is alleviated when Myc is overexpressed in the same cells. Figure 1*b* shows that cotransfection of c-Myc with Max or Max9 leads to eightfold and twofold increases, respectively, above the repressed levels induced by Max and Max9 alone. The extent of reversal of Max repression is dependent on the concentration of c-Myc expression vector (Figs 2*d* and 3*a*). Similar results (not shown) were obtained with a MinCAT reporter bearing a twofold repeat of the CACGTG-binding site, and with one carrying a fourfold repeat of the sequence CATGTG, a site also recognized by Myc–Max complexes[15]. However, a reporter gene with a fourfold MyoD target sequence (CACCTG) is not activated by Myc, and MyoD likewise fails to activate the M4MinCAT reporter (not shown), demonstrating the sequence and protein specificity of this assay.

Three c-*myc* deletion mutants (Fig. 1*a*) were also examined for their effects on Max-induced repression. The ΔC89-Myc mutant lacks the bHLH-Z domain of Myc and fails to bind Max or DNA[5]. The inability of ΔC89-Myc to affect reporter activity in the presence of Max9 (Fig. 3*d*) suggests heterodimerization is required for relief of Max repression. The ΔBR-Myc mutant forms complexes with Max that lack DNA-binding activity[5]. Introduction of ΔBR-Myc in the presence or absence of Max9 leads to an increase in reporter gene activity (Fig. 3*c* and data not shown). We presume that ΔBR-Myc acts as a dominant negative inhibitor of Max by forming non-DNA-



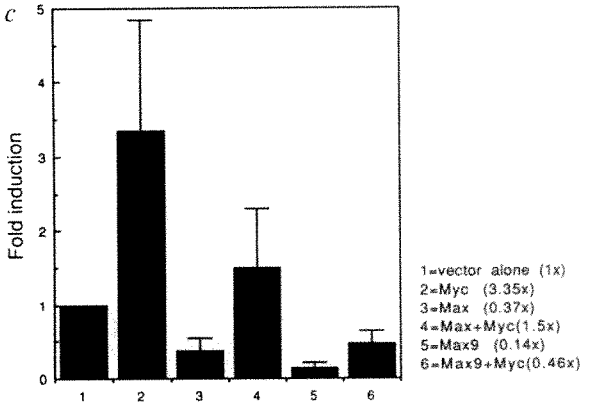




FIG. 1 Responses of the MinCAT reporter gene (+/−M4) in CV-1 cells cotransfected with Myc and/or Max vectors. *a*, Summary of constructs. MinCAT fuses chloramphenicol acetyl transferase (CAT) coding sequences to a minimal promoter (TATA box and transcription start site of the herpes simplex virus (HSV) thymidine kinase (TK) promoter). In M4MinCAT, a 4× repeat of the Myc/Max-binding site, CACGTG, was inserted proximal to this (see below). Expression vectors were derived from pEMSV (mouse sarcoma virus (MSV) long terminal repeat (LTR) promoter[22]) or pSP (simian virus 40 (SV40) early promoter; gift of M. Emmerman), which gave similar results. The *max* gene encodes protein p21; the *max*9 gene, encoding protein p22, contains 27 additional base pairs[5]. ΔBRmax lacks residues 24–36 of Max and those corresponding in Max9. Myc mutants are as described[5]. Key structural features: act, activation domain; BR, basic region; HLH-Z, helix–loop–helix–zipper domain; pA, polyadenylation signal. *b*, Reporter responses to Myc and/or Max expression. Shown are CAT assay results from duplicate plates (~$10^6$ CV-1 cells each) transfected with 10 μg of the indicated vectors and 3 μg MinCAT (−/+M4). Transfections were normalized for amount of expression vector DNA per plate and for transfection efficiencies (see below). Cells were collected ~36 h after calcium phosphate transfection, and CAT assays done[23]. CAT activity (per cent acetylated chloramphenicol, Ac-Cm) in the absence of exogenous Myc or Max defines the endogenous level of transcriptional activity, or 1×(5% Ac-Cm here). Fold inductions shown are averages of each pair relative to endogenous. *c*, Summary of results from six experiments in duplicate ($n=12$) with CV-1 and NIH3T3 cells. Endogenous levels of reporter activity, ranging from 2–10% Ac-Cm, were set to 1× in each experiment. The graph thus gives the average fold inductions in multiple experiments. In the absence of M4, Ac-Cm was well under 1%.

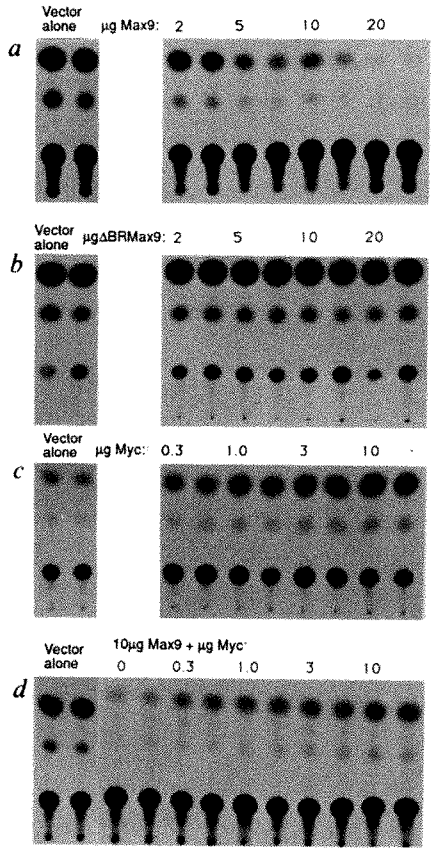
METHODS. The 4×Myc/Max-binding site was synthesized with *Sal*I ends. The sequence of one repeating unit is: CCCACCACGTGGTGCCT. Although minor variations outside the core site bound Myc with greater affinity in one report[24], we find no such difference and therefore use our originally proposed highest affinity site[9]. The MinCAT reporter construct, pGLCAT4 in ref. 25, contains nucleotides −32 to +51 of the HSV-TK gene, and M4 was ligated 15 bases 5′ of this. Myc and Max mutants, made by standard *in vitro* methods (Amersham), were sequence-verified. Overexpression of exogenous Myc and Max was confirmed by immunoprecipitation. Relative transfection efficiencies were determined by inclusion of a β-galactosidase expression plasmid (pCH110, Pharmacia LKB) and subsequent β-galactosidase assays[26].

binding complexes, partially restoring endogenous levels of transcription.

To examine whether the putative transactivation segment of Myc[4] contributes to abrogation of Max repression we used ΔN100-Myc, a mutant lacking most of the transactivating region, but containing the bHLH-Z domain. Because Max does not possess transcriptional activation capability in these and similar assays using chimaeric constructs (Figs 1 and 2; ref. 15), ΔN100-Myc and Max should form heterocomplexes capable of binding DNA[7] but lacking transactivating activity. Indeed, ΔN100-Myc has little effect on Max9 repression of the M4MinCAT reporter (Fig. 3*b*).

The results presented here suggest Max–Max and Myc–Max complexes have distinct transcriptional functions. Myc and Max differ in several important biochemical properties: Myc is a highly unstable protein[16,17] whose synthesis is rapidly induced upon cell-cycle entry[18,19] and maintained at a constant level in proliferating cells[10]. By contrast, Max is highly stable and is expressed at equivalent levels in both resting and proliferating cells[7]. Because Max normally seems to be in excess of Myc *in vivo* and Myc's short half-life is unaltered by dimerization with Max, then the rate of Myc synthesis determines the ratio of Max–Max to Myc–Max complexes[7]. These considerations lead to the scheme in Fig. 4, where relative levels of Myc–Max and Max–Max dimers are presumed to have functional consequences. Our results show that Max overexpression represses endogenous transcription from a reporter gene dependent on both a Myc/Max-binding site and the DNA-binding domain of Max. This repressive effect is abrogated by overexpression of Myc. Both these findings are consistent with observations that Myc contains, but Max lacks, a transactivation domain[15].

Extrapolation of these findings to the roles of Myc and Max *in vivo* requires caution. The promoters and regulatory circuitry of actual Myc/Max target genes are expected to be more complex than the artificial promoter used here, and other CACGTG-binding proteins, such as USF and TFE3 (refs 11, 12), may affect, and be affected by, Myc/Max expression. Furthermore



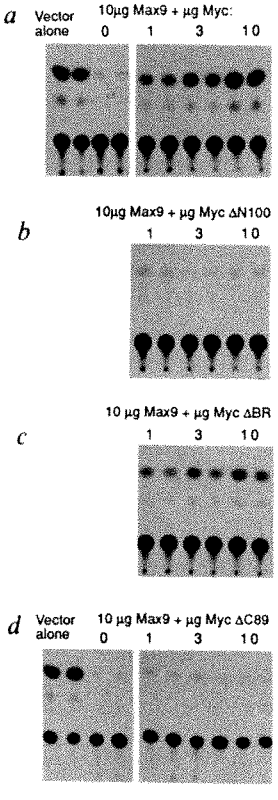




FIG. 3 Titrations of Myc mutants with a constant amount of Max9 in NIH3T3 cells. Mutants are described in Fig. 1*a* and the text. All genes expressed from the pSP vector. Endogenous activity (vector alone), 9% Ac-Cm. Fold inductions: 0.1 × for Max9 alone; 0.26 × to 0.92 × for Myc; 0.06 × to 0.13 × for ΔN100Myc; 0.27 × to 0.52 × for ΔBRMyc; and 0.2 × to 0.12 × for ΔC89Myc.

◀ FIG. 2 Myc and Max titrations in NIH3T3 cells. Increasing Myc or Max expression affects reporter activity linearly over a 10-fold range of amounts of DNA transfected. Representative experiments show endogenous activity (vector alone) with increasing amounts of Myc or Max, as indicated, following in duplicate. All methods, inlcuding normalizations, are as in Fig. 1. *a*, pEMSV-*max*9 titration. Endogenous, 5% Ac-Cm. Fold inductions are 0.78 × at 2 μg to 0.05 × at 20 μg. Similar though less strong responses are seen with *max*. *b*, Max repression requires the Max basic region. pSPΔBR*max*9 titration is shown; identical results were obtained with pSPΔBR*max*. *c*, Reporter response to Myc is concentration-dependent. Endogenous level, 9% Ac-Cm; fold inductions are 1.7 × for 0.3 μg to 4.5 × with 10 μg pSP*myc*. *d*, Myc relieves Max repression in a titratable manner. Increasing amounts of pSP*myc* were contransfected with a constant amount of pSP*max*9; similar results were seen with *myc* and *max*. Endogenous activity, 9% Ac-Cm; fold inductions are 0.13 × with no *myc*, 0.18 × with 0.3 μg *myc*, and 0.3 × with 10 μg *myc*.

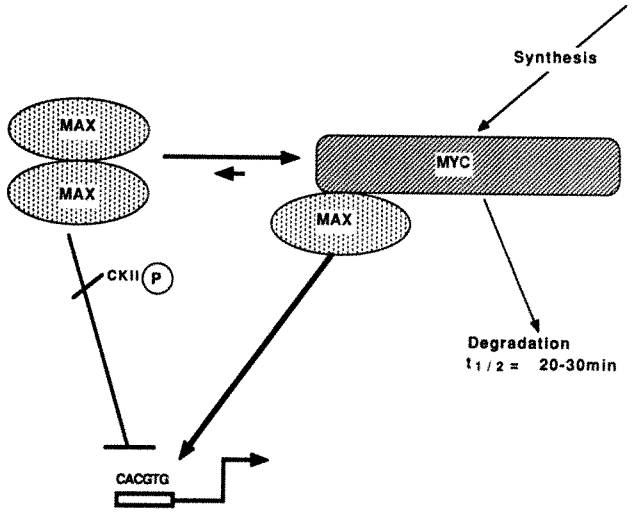



FIG. 4 Max homodimers repress, whereas Myc–Max heterodimers activate, transcription. The balance of activation versus repression of the M4MinCAT reporter reflects the equilibrium between Myc–Max and Max–Max dimers, respectively. The endogenous level of activity reflects the effects of native Myc, Max and other CACGTG-binding factors[11,12]. Max overexpression drives formation of repressive Max homodimers, whereas Myc overexpression increases the level of activating heterodimers. Further modulation of these effects is likely, such as the negative effect of phosphorylation on Max homodimer DNA binding[27].

we have found that introduction of higher levels of wild-type and mutant Myc produces 'squelching' of reporter activity, presumably due to titration of other cellular factors involved in transcription[20,21]. Nonetheless, our data, together with other studies[4,15], reveal intrinsic activities of these proteins and have implications for their function in cell proliferation. The induction of Myc upon mitogenic stimulation and the subsequent formation of Myc–Max heterodimers may activate promoters under transcriptional repression by constitutively expressed Max homodimers (Fig. 4). Furthermore, the many alterations at the c-*myc* locus in different tumours primarily lead to constitutive overexpression of Myc. Such deregulation may result in a shift from Max homodimers to Myc–Max heterocomplexes and in the enforced activation of genes normally modulated during growth and differentiation. □

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# Truncation variants of peptides isolated from MHC class II molecules suggest sequence motifs

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**T CELLS recognize foreign protein antigens in the form of peptide fragments bound tightly to the outer aspect of molecules encoded by the major histocompatibility complex (MHC). Most of the amino-acid differences that distinguish MHC allelic variants line the peptide-binding cleft, and different allelic forms of MHC molecules bind distinct peptides[1,2]. It has been demonstrated that peptide-binding to MHC class I involves anchor residues in certain positions and that antigenic peptides associated with MHC class I exhibit allele-specific structural motifs[3]. We have previously reported an analysis of MHC class II-associated peptide sequences[4]. Here we extend this analysis and show that certain amino-acid residues occur at particular positions in the sequence of peptides binding to a given MHC class II molecule. These sequence motifs require the amino terminus to be shifted one or two positions to obtain alignment; such shifts occur naturally for a single peptide sequence without qualitatively altering CD4 T-cell recognition.**

We previously identified the sequences of 12 naturally processed peptides associated with I-$A^b$ and I-$E^b$ (ref. 4). To assess allele- and isotype-specific sequence motifs in MHC class II-associated peptides, we have isolated and sequenced peptides from I-$A^s$ and more peptides from I-$A^b$ and I-$E^b$. As in our previous study, the MHC class II I-$A^s$ molecule was affinity-purified from a B lymphoma cell line LS102.9 (ref. 5). The purified I-$A^s$ molecules run as a single band on silver-stained SDS-polyacrylamide gels (not shown). The peptides eluted from I-$A^s$ in 2.5 M acetic acid were analysed by reversed-phase high-performance liquid chromatography (RPHPLC), giving a pattern of peaks distinct from those observed with purified I-$A^b$ or I-$E^b$ (Fig. 1; ref. 4). Several of these peaks were analysed by Edman degradation and yielded clear sequences (Table 1). As previously observed, the peptides were around 16 amino-acid residues long, were derived from proteins that recycle through the endocytic compartment, and peptides differing only by one or more amino acids in length appeared in distinct HPLC peaks. Two peptides were derived from the transferrin receptor, one peptide each from CH1 and CH2 domains of IgG2a, and two peptides from a murine leukaemia virus envelope (MuLV env) protein. We previously obtained several peptides of distinct length from what seems to be the same MuLV env protein (S.-C. Hong, A.Y.R. and C.A.J., unpublished data).

Further analysis of peptides eluted from I-$A^b$ in LB27.4 cells[4] has identified peptides A$\beta$55–66, E$\alpha$52–66, and a peptide apparently derived from the immunoglobulin VH region (residues 59–74) spanning part of CDR2 and FR3 (~80% identity to a number of VH(IIB)) (Table 2). The three immunoglobulin peptides derived from I-$A^s$ and I-$A^b$ are examples of tissue-specific peptide presentation and provide a structural basis for earlier observations on very efficient B-cell processing and presentation of endogenous immunoglobulin by MHC class II (refs 6–8). Two more peptides, MuLV env protein 454–475 (SPSYVYHQFERRAKYKREPVSL, in single-letter amino-acid code) and BSA 141–157 (GKYLYEIARRHPYFYAP), have been isolated from I-$E^b$. These peptides are identical to those previously described[4] except at the carboxy terminus.

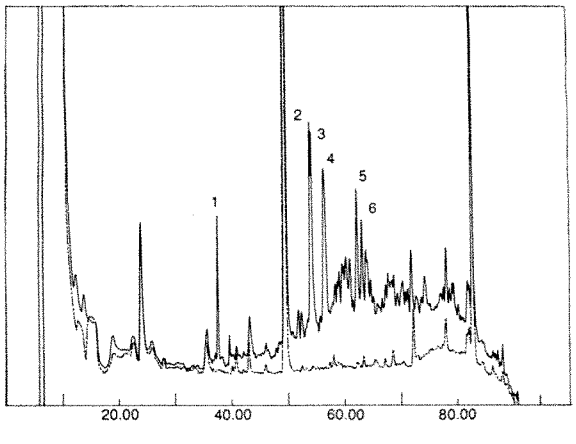



FIG. 1 RPHPLC profile of peptides eluted from I-$A^s$. C18 column HPLC separations of peptides acid-eluted from the purified I-$A^s$ (solid line) as well as control eluates from bovine serum albumin (dotted line).
METHODS. LS102.9 hybrid B lymphoma cells[5] were grown in EHAA medium supplemented with 5% heat-inactivated donor calf serum and 50 $\mu$g $ml^{-1}$ gentamycin. I-$A^s$ molecules were purified from cell lysate by affinity chromatography on Y-3P anti-I-$A^{b/s}$ monoclonal antibody columns as described[4]. The purification was monitored by SDS-PAGE and sandwich ELISA for I-$A^s$ as described[4,26] (data not shown). Peptides were acid-eluted from 500 $\mu$g acetonitrile-precipitated I-$A^s$ and BSA (negative control) and further separated by RPHPLC on a Vydac C18 column (250 × 4.6 mm 300 Å, 5 $\mu$m) using a Waters HPLC system as described[4]. Buffer A, 0.06% TFA, $H_2O$; buffer B, 0.052% TFA, 80% acetonitrile. Gradient: 0–60 min, 2–37.5% B; 60–90 min, 37.5–75% B; 90–105 min, 75–98% B. Injection volume, 200 $\mu$l. Absorbance of fractions was measured at 210 nm, shown as absorbance units at full scale of 0.035. Flow rate, 0.5 ml $min^{-1}$. Fractions were collected and the material in main peaks in the figure were sequenced on an Applied Biosystems 477 gas phase protein sequencer. The amino-acid sequence obtained were compared with known proteins in the GenBank database using the FASTA program[27]. Only instances of 100% identity with a known sequence are attributed.

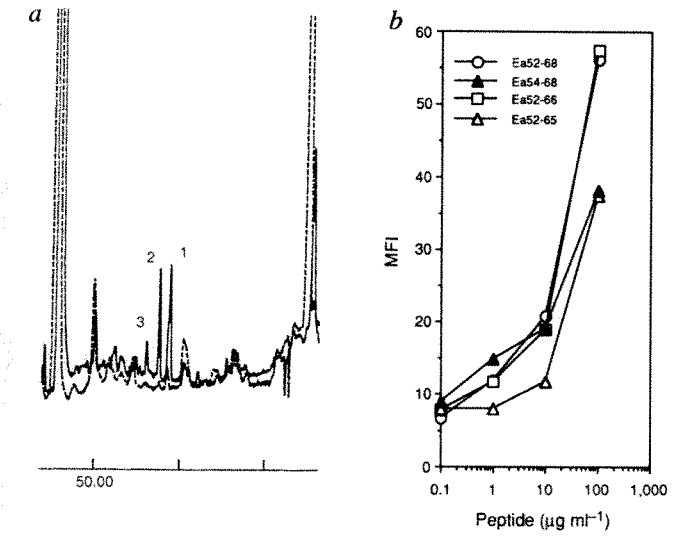
TABLE 1 Sequences of I-$A^s$-associated peptides

| Peptide donor | Peptide sequence | Position | Length | HPLC fraction |
|---|---|---|---|---|
| IgG2a | NVEVHTAQTQTHREDY* | 281–296 | 16 | 1 (24) |
| Transferrin receptor | KPTEVSGKLVHANFGT | 203–218 | 16 | 2 (42) |
| Transferrin receptor | KPTEVSGKLVHANFGx | 203–217 | 16 | 3 (43) |
| IgG2a | WPSQSITCNVAHPASST* | 194–210 | 17 | 4 (46) |
| MuLV envelope protein | IRLKITDSGPRVPIGpn | 255–269 | 15 | 5 (56) |
| MuLV envelope protein | IRLKITDSGPRVP | 255–267 | 13 | 6 (57) |
| Secondary | XPYMFADKVVHLPGSQ | — | 16 | 6 (57) |

The peaks numbered sequentially in Fig. 1 are arranged by sequence here, as noted. Actual fraction number is shown in parentheses. C termini of peptides we could not identify with confidence are shown in lower case. The precise murine leukaemia virus from which these peptides derive is unknown; it appears to be similar to but distinct from both RadLV and AKV (S.-C. Hong, A.Y.R. and C.A.J., unpublished results). The numbering used for MuLV env peptides corresponds to the AKV sequence.
* EV index[9].

The variability at the C termini and the wide range of length (from 12 to 22 residues) seems to be a general feature of MHC class II-associated peptides isolated from B-lymphoma cells and is in contrast to peptides derived from MHC class I molecules. The observations raise the following important question: is similar variability characteristic of *in vivo*-derived peptides and, if so, can such naturally processed peptides of distinct length but having an identical core sequence be distinguished by T cells? Previously, one particularly prominent I-$A^b$-binding peptide was identified as E$\alpha$52–68 (earlier called E$\alpha$56–73; the current numbering is from ref. 9). This peptide, bound to I-$A^b$ molecules, is recognized by the Y-A*e* monoclonal antibody as well as by T-cell hybrids raised against E$\alpha$52–68 in C57BL/6 (H-$2^b$) mice[10]. This complex accounts for about one eighth of surface I-$A^b$ molecules in the I-$E^+$ strain B10.A(5R). To answer the above question, we have analysed E$\alpha$-related peptides isolated from I-$A^b$ molecules affinity-purified from spleen cells of ~100 B10.A(5R) mice. Three prominent peaks observed in the HPLC analysis consisted of E$\alpha$52–68, E$\alpha$52–66 and E$\alpha$54–67 peptides in order of decreasing yield (Fig. 2*a*). Thus, variability in peptide length is observed *in vivo* and, in addition to the C-terminal variation observed in this and our previous study, variability in the N terminus of the same peptide can also occur. The above data and our preliminary studies on the binding of E$\alpha$ peptide extended on the N terminus support the idea[11] that MHC class II molecules have peptide clefts open at both ends, and that peptides can extend in either direction from a central core region.

To assess the immunological consequences of these variations in peptide length, synthetic E$\alpha$ peptides truncated at the N or C terminus were added to H-$2^b$ spleen cells, and the binding of Y-A*e* to these cells was measured (Fig. 2*b*). Truncation of two N-terminal amino acids still allowed strong binding of Y-A*e*, as did truncation of two or three C-terminal residues. Moreover, responses of a series of nine independent E$\alpha$52–68-specific, I-$A^b$-restricted T-cell hybrids showed strong responses to E$\alpha$54–68, E$\alpha$52–66, and E$\alpha$52–65, comparable to that elicited by E$\alpha$52–68 (Fig. 2*c* and data not shown). Similar results are obtained using fixed spleen cells and serum-free medium. Thus,



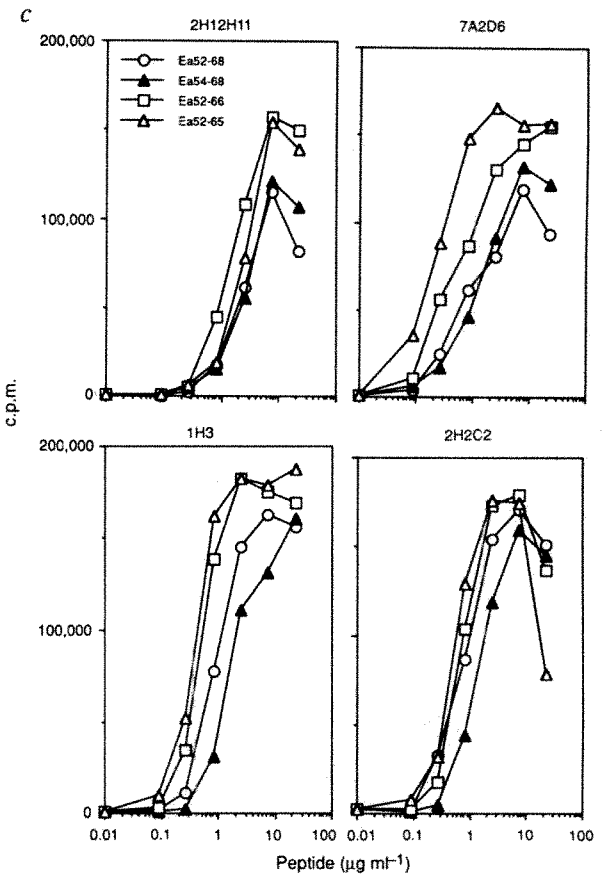




FIG. 2 Naturally occurring variants of E$\alpha$52–68 peptides. RPHPLC profile and amino-acid sequences of peptides eluted from I-$A^b$ purified from B10.A(5R) spleen cells (solid line) and BSA (dotted line) (*a*). FACS analysis of Y-A*e* binding (*b*) and T-cell hybrid responses to synthetic truncation variants of the E$\alpha$52–68 peptide (*c*).
METHODS. I-$A^b$ (500 μg) was purified from spleens of 100 B10.A(5R) mice, peptides eluted, separated on C18 RPHPLC column and sequenced as described in Fig. 1. Absorbance of fractions was measured at 210 nm, shown as absorbance units at full scale = 0.010. Peptides were synthesized on an Applied Biosystems solid-phase peptide synthesizer using t-BOC chemistry. All peptides were purified by RPHPLC and the identity was verified by amino-acid analysis and mass-spectrometry. B-cell LPS blasts from C57BL/6 mice were pulsed with E$\alpha$ peptides at 100, 10 and 1 μg $ml^{-1}$ for 2.5 h and after washing, binding of Y-A*e* antibody was analysed by FACS as described[9]. Data are presented as mean fluorescence intensity. The production of interleukin-2 by E$\alpha$52–68:I-$A^b$-specific T-cell hybrids has been described before[10]. Interleukin-2 production was monitored using a standard CTLL assay.

TABLE 2 Sequence motifs in naturally processed MHC class II-associated peptides

| MHC molecule | Peptide | Peptide sequence |
|---|---|---|
| I-A$^s$ | Consensus | X X X X **I T** X X X X **H** X X X |
| | MuLV env 255–269* | I R L K **I T** D S G P **R** V P I G p n |
| | IgG2a 194–210*§ | W P S Q S **I T** C N V A **H** P A S S T |
| | IgG2a 281–296*§ | N V E V H **T A** Q T Q T **H** R E D Y |
| | TfR 203–218* | K P T E **V S** G K L V **H** A N F G T |
| | Undefined* | X P Y M **F A** D K V V **H** L P G S Q |
| I-A$^b$ | Consensus | X X **N** X X X X X **P** X X X X |
| | MuLV env 145–158† | H **N** EGF Y V C **P** G P H R P |
| | E$\alpha$ 52–68† | A S F E A **Q** G A L A N **I** A V D K A |
| | Ii 85–99† | K P V S **Q** M R M A T **P** L L M R |
| | A$\beta$ 55–66* | R P **D** A E Y W N **S** Q P E |
| | IgGVH 59–74* | X **N** A D F K T **P** A T L T V D k p |
| | Undefined† | N Y **N** A Y N A T **P** A T L A V D |
| I-E$^b$ | Consensus | X X **Y L Y** X X X X **R R** X X **Y** X |
| | MuLV env 454–469† | S P S **Y V Y** H Q F E **R R** A K **Y** K |
| | BSA 141–157*† | G K **Y L Y** E I A R **R H** P **Y F** y a p |
| | Undefined† | X P Q S **Y L I** H E X X X I S |
| | DR consensus‡ | A A **Y** A A A A A A A **K** A A A |

The sequences shown above have been identified in this paper (*) or in ref. 4 (†) and 17 (‡). Typical residues found in several of the peptides are shown in bold; these may represent residues involved in peptide-binding to the relevant MHC class II molecule. To obtain optimal alignment of the peptides, variation by plus or minus two residues at the N terminus were introduced, on the basis of the finding of such truncations occurring naturally without preventing peptide recognition by T cells (see text). In addition, the I-A$^b$-binding MuLV env 145–158 peptide was compressed around a glycine residue. This change is based on the finding that both 8 and 9 amino-acid peptides bind to the MHC class I molecule K$^b$, and that this occurs by a kink at a proline residue in the longer peptide, allowing its ends to bind appropriately[28,29]. Only direct binding data of modified peptides can guide the identification of correct assignments in individual peptides.

§ EV index[9].

N- and C-terminal truncations of the same peptide occur in normal cells, and these do not prevent T- or B-cell recognition of the peptide/MHC class II complex when these differences are of two amino acids or less. A preliminary analysis of single amino-acid interchanges in E$\alpha$54–66 suggests that only the middle part of the peptide QGALANIA is critical for recognition by most T cells and the Y-A*e* antibody (A.Y.R., J.R. and C.A.J., manuscript in preparation). As T cells seem not to discriminate qualitatively between peptides of distinct length but identical core sequence, the differences in peptide length should not contribute notably to the complexity of self MHC class II ligands to which T cells must be tolerant.

Peptides eluted from MHC class I molecules have a defined length of 8 or 9 amino acids depending on the MHC class I molecule examined[12–14]. Moreover, specific amino-acid residues are found at one, two or three positions in the sequence, giving distinctive sequence motifs for peptides binding to each MHC class I molecule[3]. The fact that an MHC class II-associated peptide can be naturally truncated both at the N and C termini suggests that shifting the amino terminus of peptide sequences by plus or minus one or two positions may be required to obtain alignment of functionally equivalent residues. Using this new information, alignment of the peptides eluted from I-A$^s$, I-A$^b$ and I-E$^b$ reveals distinctive sequence motifs in which the same or structurally related amino-acid residues occur at particular positions in most or all of the sequences (Table 2). Sequence motifs requiring similar alignment have also been observed in peptides eluted from I-A$^d$ where N- and C-terminal truncations have also been observed and shown not to affect peptide-binding[15]. This suggests that the pooled peptide sequencing method[3] to define MHC class I-associated peptide motifs will be difficult to apply to MHC class II-associated peptides. The sequence motif identified for I-E$^b$-derived peptides can be found in known I-E-binding peptides[2,16] and is similar to a common motif determined by the binding of synthetic peptides to purified HLA-DR molecules[17,18], yet is different from the motifs of peptides derived from I-A$^s$ and I-A$^b$ (refs 19–24), suggesting that there may be MHC isotype specificity of peptide-binding in addition to allele specificity. This indicates that MHC class II isotypes may allow presentation of different classes of peptides and may be selected on this basis. Some of the residues that define these motifs are likely to be involved in anchoring the peptide to MHC class II molecules, as has been demonstrated in the case of MHC class I molecules[3,25]. Preliminary studies of peptide binding to I-A$^s$ and I-A$^b$ support this conclusion. □

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# Probing *met* repressor–operator recognition in solution

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**THE three-dimensional crystal structure of the *Escherichia coli* methionine repressor, MetJ, complexed with a DNA operator fragment is described in an accompanying article[1]. The complex exhibits several novel features of DNA–protein interaction. DNA sequence recognition is achieved largely by hydrogen-bond contacts between the bases and amino-acid side chains located on a $\beta$-ribbon, a mode of recognition previously hypothesized on the basis of modelling of idealized $\beta$-strands and DNA[2], and mutagenesis of the *Salmonella* phage P22 repressors Arc and Mnt[3]. The complex comprises a pair of MetJ repressor dimers which bind to**

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**adjacent *met*-box sites on the DNA, and contact each other by means of a pair of antiparallel $\alpha$-helices. Here we assess the importance of these contacts, and also of contacts that would be made between the C-helices of the protein and DNA in a previous model of the complex[4], by studying mutations aimed at disrupting them. The role of the carboxy-terminal helix face in operator binding was unclear, but we demonstrate that recognition of operator sequences occurs through side chains in the $\beta$-strand motif and that dimer–dimer interactions are required for effective repression.**

We produced mutations in the MetJ subunit to disrupt the contacts for DNA binding or the interaction between dimers (Fig. 1). The mutant proteins were screened *in vitro* using a gel retardation assay[5] (Fig. 2) and *in vivo* using the assay described in Table 1, where the results are listed. Mutations in the $\beta$-strand DNA-binding motif fall into three groups, corresponding to changes at three residues. These are mutations of Lys 22 (to Glu, Ala or Arg), whose side chain in the crystal structure makes contact with the phosphate backbone of the DNA; Lys 23 (to Glu, Ala or Arg), which is hydrogen-bonded to the guanine residue at operator positions 2, 7, 10 and 15 (see Table 1 and ref. 1 for details of numbering system); and Thr 25 (to Val, Gln or Asn), which is hydrogen-bonded to N7 of the adenines at positions 3, 6, 11 and 14 of the operator. The data for Lys 23 and Thr 25 are consistent with both residues being involved in sequence-specific recognition. Mutations at Lys 22 suggest slight deleterious effects on operator recognition *in vitro* of the Glu and Ala mutations, with somewhat better than wild-type affinity for Arg substitution. But *in vivo* these mutants are all wild-type

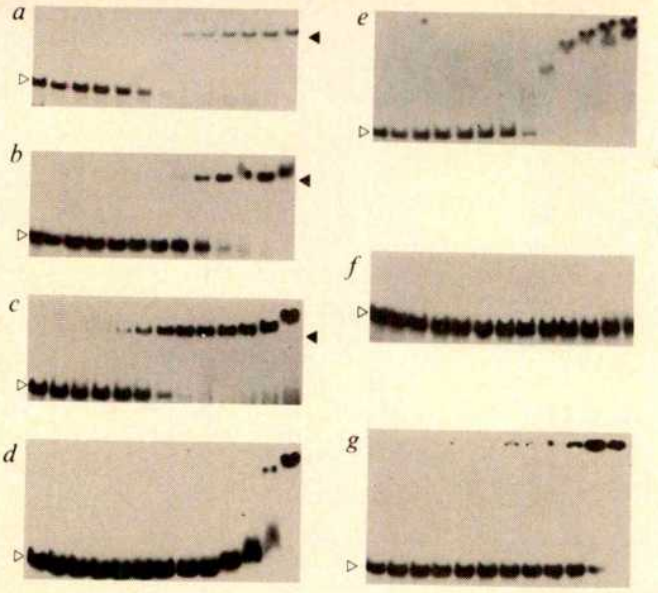


FIG. 2 Results of gel retardation assays using several of the mutant MetJ repressors. Open arrowheads indicate the position of free operator-fragment DNA, solid arrowheads mark the position of sequence-specific retarded complexes. Samples were diluted serially, twofold from right to left. Samples (and initial protein concentrations) were as follows: *a*, wild-type MetJ (2.28 μM), *b*, Q94R (1.47 μM), *c*, K22R (4.83 μM), *d*, K23R (10 μM), *e*, T25N (8.95 μM), *f*, T37A (13.33 μM), *g*, R40E (170 μM).

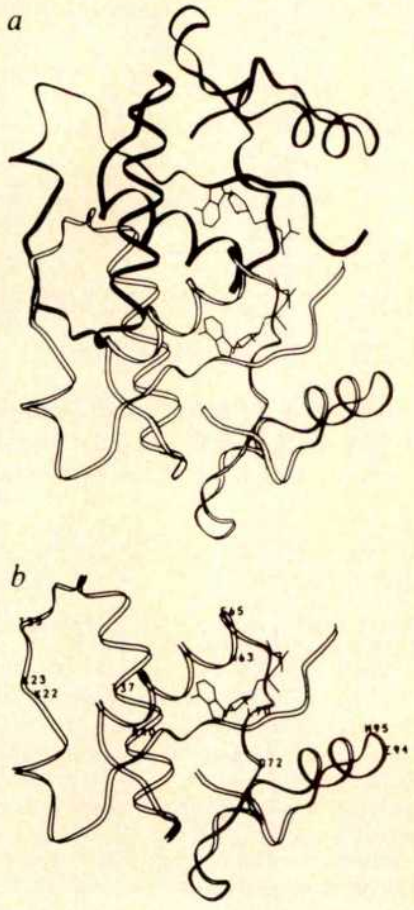


FIG. 1 *a*, Ribbon representation of the backbone of a MetJ dimer in the conformation adopted when bound to DNA. One subunit is shaded and the corepressor is shown as a stick model. The DNA binds to the $\beta$-strands on the left-hand face of the molecule in this view. *b*, A single subunit of the dimer showing the locations of the residues mutated in this study.

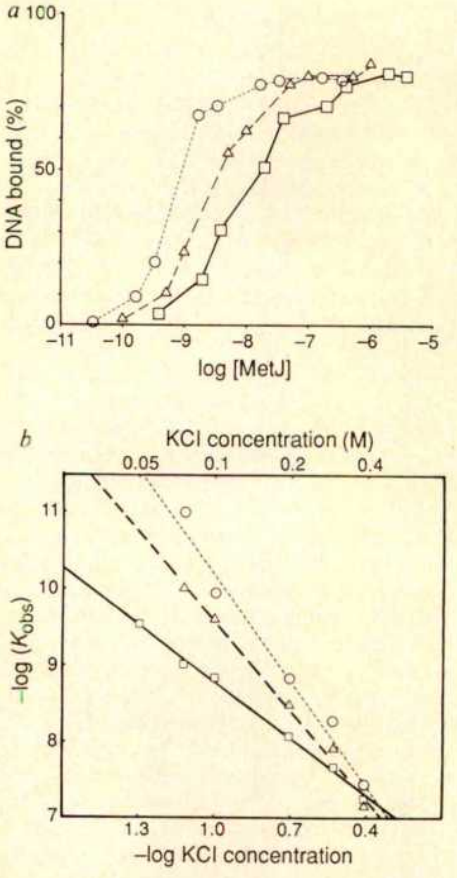


FIG. 3 *a*, Nitrocellulose filter-binding assays of purified mutant repressors. Assays were carried out using a radiolabelled restriction fragment containing the anti-*met*-box construct[5] (00186). Wild type (□); Glu 94 → Arg (△); Asp 72 → Arg (○). *b*, Salt-dependence of operator binding. Equilibrium binding experiments were done under standard assay conditions. The ionic strength of the binding buffer was changed by altering the KCl concentration. Labels as in *a*. Lines are the linear regressions through the data.

TABLE 1 Results of *in vitro* and *in vivo* binding assays for the MetJ mutants

| Mutant protein | Relative affinity *in vitro* ($K_d$s) | Relative repression ratios *in vivo* |
|---|---|---|
| WT* | 1 | 1.0 |
| β-strand DNA-binding motif | | |
| K22E | 3 | 0.7 |
| K22A | 3 | 0.9 |
| K22R | <1 | 0.2 |
| K23E | 8 | 132.0 |
| K23A | 30 | 124.0 |
| K23R* | 431 | 4.8 |
| T25V | 61 | 110.0 |
| T25Q | 34 | 134.0 |
| T25N* | 27 | 128.0 |
| Protein-protein or protein-DNA backbone interactions | | |
| T37Y | 91 | 94.0 |
| T37A* | >1,000 | 99.0 |
| R40E* | >1,000 | 20.0 |
| SAM-binding site | | |
| L70R | 9 | 72.0 |
| H63Q | 15 | 96.0 |
| F65A | 13 | 0.3 |
| C-helix face | | |
| M95W | 3 | 0.8 |
| E94R* | 6 | 0.7 |
| D72R* | 5 | 0.6 |

The *in vivo* data are the ratios of β-galactosidase activities in *E. coli* GT1008 (metJ$^-$) carrying two plasmids: the metF'-lacZ$^+$ reporter construct pIP86 (ref. 6) and either pGW11 (control) or pGW11-tacmetJ (metJ$^+$). The β-galactosidase activity of the wild-type repressor results in the production of 37.6 nmol of *O*-nitrophenol per min, per mg protein at 28 °C, pH 7.0. The DNA substrate for operator binding *in vitro* is the anti-*met*-box construct, designed to assay binding of repressor to two, tandem 8 bp *met*-box sites[5]. Relative affinities were determined in gel retardation assays and were normalized to a wild-type (WT) $K_d$ of 1. The sequence numbering of the *met* operator is as the description of the DNA fragment in the crystal[1], where bases 1–8 and 9–16 correspond to consensus *met* boxes

```
     -1 0  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17
5'-  T  T  A  G  A  C  G  T  C  T  A  G  A  C  G  T  C  T  A  .  -3'
3'-  .  A  T  C  T  G  C  A  G  A  T  C  T  G  C  A  G  A  T  T  -5'
```

Site-directed mutagenesis was done using the phosphothioate method (Amersham). Cloned mutated genes were fully sequenced to ensure that the only changes were those required. The DNA fragments were then subcloned into an expression vector and overexpressed in *E. coli* GT1008 (metJ185am)[6]. Cell extracts were prepared by sonication, treated with DNaseI to remove nucleic acids and the *met* repressor concentration estimated using a combination of the Bradford assay and SDS–PAGE. Although the extent of expression varied between different mutant proteins the extracts contained between 20 and 50% of total protein as *met* repressor. Several of the mutants have been purified to homogeneity (indicated by *) and used in gel retardation assays. The values for operator affinities determined for these mutants were identical (within experimental error) to those determined in the extracts. Native gels, circular dichroism spectra and differential scanning calorimetry (C. M. Johnson, personal communication) of purified mutant proteins show that the mutations have little or no effect on the stability and folding of the dimers, suggesting that changes in binding affinity result only from elimination of the intended intermolecular contacts. Where possible the purified proteins have been sequenced at the amino terminus to confirm the presence of the expected mutation. Complexes were formed between protein and DNA in the presence of the corepressor, SAM (1 mM) at 37 °C for 15 min before electrophoresis in 10% (w/v) polyacrylamide gels containing 0.1 mM SAM. The affinity of each repressor mutant was estimated by serial twofold dilution of the cell extracts across the range where retardation of the DNA fragments was observed. Autoradiographs of the gels were then analysed by densitometry and the *met* repressor protein concentration at which 50% of the input DNA was retarded from its unshifted position was estimated.

within experimental error, suggesting that this contact does not contribute significantly to the overall stability of the operator complex.

Mutations in the A-helices, which mediate the interaction between adjacent repressors, were at two positions, Thr 37 in the dimer–dimer interface and Arg 40 which makes a contact with the DNA phosphate backbone. The results suggest that both contacts are important for effective repression, confirming the requirement for protein–protein cooperativity in operator binding[5,6].

Mutations that affect corepressor binding are of two types. The first includes residues that contact the bound corepressor, *S*-adenosylmethionine (SAM), directly (His 63 and Leu 70). Replacement of these side chains, by Gln and Arg, respectively, results in significantly lower affinities for operators both *in vitro* and *in vivo*, presumably by lowering the affinity for SAM, although the $K_d$s for SAM binding were not determined. The second type of mutation is at Phe 65, the side chain that must rotate out of a hydrophobic pocket in the apoprotein to allow SAM to bind[4]. Replacement by Ala has a deleterious effect *in vitro* but essentially acts as the wild type *in vivo*.

Three mutations directed at the C-helix face of the protein have also been analysed. Met 95 to Trp substitution yields a slightly lower affinity for the operator fragment than wild type *in vitro*, but essentially acts as the wild type *in vivo*. Mutation of residues Glu 94 (to Arg) and Asp 72 (to Arg) yield unexpected behaviour. In the *in vivo* assay both mutations behaved as the wild type, but they had slightly lower affinities than wild type *in vitro* as judged by gel retardation assays. But in nitrocellulose filter-binding assays, both mutant proteins apparently had increased affinities for DNA compared with the wild-type protein, and altered ionic strength dependencies of operator affinities (Fig. 3). We believe that the differences in affinities determined by the different techniques may be due to the relatively rapid sampling of the filter-binding assay. This could trap transient complexes, which would have time to dissociate during electrophoresis into the gel at the start of a gel retardation experiment. Both mutations result in a net increase of four positive charges per repressor dimer, which is consistent with the observed increase in electrostatic attraction to the DNA.

Clearly more experiments are required to probe the role, if any, of the C-terminal helix face in operator binding. But the results with the other MetJ mutations demonstrate that side chains in the β-strand motif mediate recognition of operator sequences, and also that dimer–dimer interaction is important for effective repression[5,6]. These data, together with the results of *in vitro* binding assays, chemical and enzymatic footprinting, and systematic variation of operator sequences (P.G.S. *et al.*, unpublished results), confirm the essential features of the repressor–operator interaction seen in the crystal structure. □

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nature genetics October

# Diabetes defect defined

**McIlraith would be pleased — one hundred years after his documentation of nephrogenic diabetes insipidus, the pursuit of the molecular pathology behind this condition has finally succeeded.**

THERE is a satisfying temporal neatness to be found in the centenary, this very month, of the first description[1] of nephrogenic diabetes insipidus (NDI). For, in the October issue of *Nature Genetics*, two groups[2,3] present a total of nine separate mutations associated with the disease. These papers reflect the quickening tempo of research.

For the past few months there has been little doubt that the defect underlying NDI, an X-linked recessive disorder, would be found within the gene for the vasopressin (antidiuretic hormone) V2 receptor[4,5]. In NDI patients, the renal tubule is insensitive to vasopressin, leading to an inability to concentrate urine despite elevated levels of the hormone. Untreated, the condition can cause severe dehydration, growth reduction and even mental retardation. The simple remedy is to ensure an adequate intake of fluids.

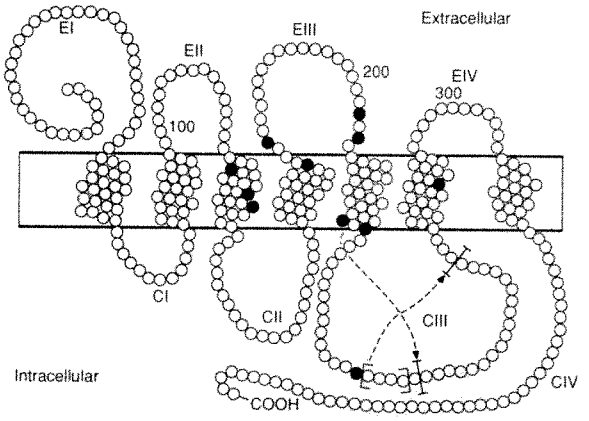
Barely four months ago, the cloning of the V2 receptor gene from human[6] and rat[7] was reported; furthermore, the human gene mapped to chromosome Xq28, making it a prime candidate for the NDI locus which had been previously assigned to the same region. Astute readers will recall that such suspicions were indeed confirmed two weeks ago with the description of mutations in the V2 receptor gene in two patients[8]. Now we have the association of nine separate mutations with the disease reported in *Nature Genetics*, and yet others have also been uncovered (see figure).

The V2 receptor is a member of the G-protein-coupled receptor family possessing the characteristic seven membrane-spanning regions[4] (see figure). Rosenthal *et al.*[8] amplified the V2 receptor gene from a severely affected Canadian patient and found a deletion of one of six consecutive guanosine nucleotides at codon 246, resulting in premature termination of the protein 23 residues downstream. A second patient had an alanine to aspartic acid substitution at codon 132 (Ala 132→Asp) in the third transmembrane domain.

As they now report, Bernard van Oost and colleagues[2] chose to concentrate on the region of the V2 receptor gene encoding the third extracellular domain (numbered according to ref. 4), thought to be important for the binding of the hormone. They found point mutations in three of eight NDI patients. Interestingly, all three extracellular mutations create new cysteine residues (from Gly 185, Arg 203 and Tyr 205) which might disrupt the tertiary structure of the receptor and interfere with vasopressin binding[2,4].

Meanwhile, Jane Gitschier's group[3] adopted single-strand conformational polymorphism analysis to search for deviations of the V2 receptor gene from the normal sequence. Five of the six patients analysed showed such abnormalities: sequencing revealed that three mutations occur within transmembrane domains (Gln 119→Stop, Tyr 128→Ser and Pro 286→ Arg); one lies at the start of the third extracellular domain and also generates a cysteine residue (Arg 181→Cys); and two changes occur in the third intracellular loop (a frameshift after Arg 230 and deletion of residues 247–250). One patient with a positive family history had two of these defects (Arg 181→Cys and the Arg 247–Gly 250 deletion). Finally, a fourth group has uncovered still more mutations — a cytosine insertion in codon 228 (Ile), also predicted to truncate the V2 receptor in the third cytoplasmic loop, and another creation of an extracellular cysteine residue (Arg 202→Cys) (A. Spiegel, personal communication).

Already, then, some notable trends are emerging. Mutations in the V2 receptor are clustered in three distinct parts of the molecule — the third transmembrane domain, the third extracellular domain (in all five cases creating cysteine residues that may interfere with disulphide bond formation) and the third cytoplasmic loop, where frameshift mutations lead to premature termination of the protein. These are predicted to produce a non-functional protein, genetically termed a *null* allele. In males, with only one copy of the X-linked gene, such (recessive) mutations confer the affected phenotype. These are not the first examples of recessive mutations in a G-protein-coupled receptor — Dryja and colleagues have described a mutation in the rhodopsin gene, which also produces a truncated protein and leads to autosomal recessive retinitis pigmentosa[9]. But the new studies do represent the first identified mutations in a G-protein-coupled hormone receptor.




Vasopressin V2 receptor mutations in NDI. Filled circles denote substitutions or frameshift mutations; arrows indicate truncated sites; brackets represent in-frame deletion. (Adapted from ref. 4.)

The new molecular data also allow examination of the so-called 'Hopewell hypothesis'. This holds that most NDI cases in the United States trace back to the Celtic settlers who landed in Nova Scotia in October 1761 aboard the ship *Hopewell* (see ref. 5), and therefore that only a small number of original mutations accounted for the disease. The hypothesis seems to have been sunk, however, with the discovery of at least 13 distinct mutations, many from families in North America[3,8]. **Kevin Davies**

*Kevin Davies is editor of* Nature Genetics.

Also in this month's *Nature Genetics*: disruption of a *trithorax*-like gene in acute childhood leukaemia; potential defect in L1CAM, a neural cell-adhesion molecule, in X-linked hydrocephalus; typing DNA from dental remains; and disorders in skeletal muscle sodium channels in humans and race horses.

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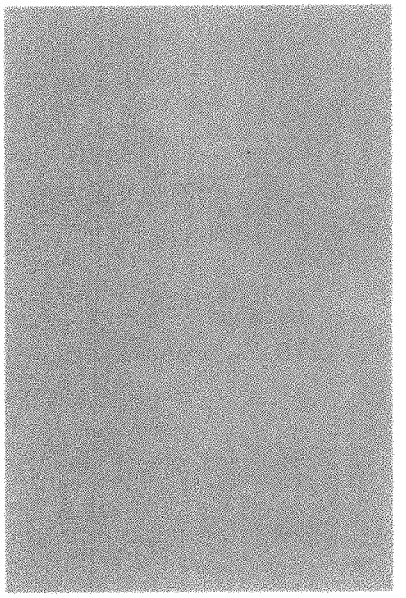

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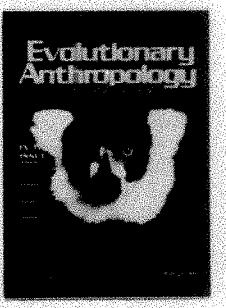


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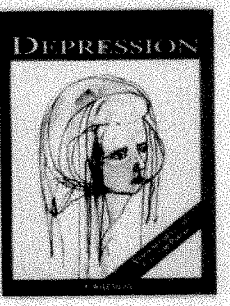


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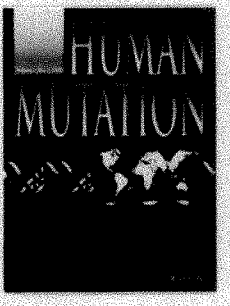


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*Phil Barden*

Electronic publishing and its twin, electronic networking, would seem to have arrived. But have they been pushed too hard, too soon?

ELECTRONIC publishing, rather like expert systems, is rapidly becoming a catch-all term. Just as expert systems were reified too early in their development, so there is also a danger of electronic publishing becoming an ill-defined enterprise, promising too much, too soon. In particular, there are several important hurdles that will have to be overcome if we are ever to establish a true electronic information system. Of course, electronic publishing can be discussed without reference to networks. But for better or worse, the medium and the message are already being identified with one another.

Electronic publishing is by no means new, and where it exists it is to a certain extent successful. Sales of CD-ROMs have grown considerably over the past two years, and commercial electronic full-text databases such as Lexis and Medline have long been earning substantial revenues. More recently, we have witnessed the advent of electronic journals published on high-speed digital communications networks. The advantages of this kind of 'global multimedia' publishing to scientists are obvious: rapid communication and response, massive capacity and powerful searchability.

The benefits are well illustrated by the electronic journal *Current Clinical Trials*, published earlier this year by the American Association for the Advancement of Science in collaboration with the leading US library network OCLC. The collaboration is only one of a growing number of similar projects emerging in the United States. In addition, there are already more than 30 journals published on US academic networks and about 100 bulletin boards for less formal exchanges of information. Some informal networks are very advanced, such as the 'World-Wide Web' global initiative conceived at CERN as an essential infrastructure for the particle physics community.

## Future plans

In the United Kingdom, the JANET network will allow users to send full-text articles to any other JANET user. And with Super-JANET, which permits a much wider range of high-speed data transmission, one will be able to send an article with good-quality graphic images not only to other JANET users, but also to the thousands of US users of the open network system Internet. The much-discussed Adonis project, which plans to publish a weekly issue of an optical disk carrying papers from the current issues of more than 400 scientific journals, is nearly complete. In the United States, the CORE project, mounted at Cornell University, will soon enter the final stage of testing. This is said to be the largest body of electronic text of its kind, containing the full text and graphics of 20 American Chemical Society journals from 1980 onwards, with associated abstracting and indexing information.

On the broader stage, the bill for the funding of the National Research and Education Network, the successor to Internet, has been voted in by the Senate in the United States. Championed by Senator Al Gore, this gigabit 'data superhighway', which is intended to link universities, colleges, schools and national research institutes, will provide the United States with a networking infrastructure that should easily see the country into the next century. In Europe, many networking bodies are attempting to untangle the knots of incompatibility of various systems, thereby making networks more effective and producing an information infrastructure that is truly European.

But these networks remain largely prescriptive; little has been done to match them to each other or to accommodate the huge diversity of customer needs. For example, academic librarians welcome electronic publishing as a way of releasing their constrained periodical budgets, thereby allowing them to provide a better service. On the other hand, individual researchers are attracted to the increased pace, power and capacity of electronic publishing and networks, and by the prospect of being freed from the shackles of a restricted library service.

Publishers are in a somewhat different position, realizing that like the atom bomb, electronic publishing cannot be dis-invented. They must respond appropriately if their revenues are not to be eroded, and have already started to form alliances with network organizations. There is little chance that publishers will be excluded altogether; they have valuable experience and skills that they can add to the publishing process, such as knowledge about the collection, presentation, organization, distribution and commercial sale of information. Finally, for groups of collaborating researchers on different continents, wideband networks will allow the rapid sharing and exchange of information, including video and sound. It is here that the need for gigabit networks becomes increasingly obvious.

And who will control and organize the information transmitted? There will have to be discussions with publishers about such awkward matters as copyright, scanning, electronic storage and transmission of hard copy; detailed examination of what the 'end user' requires; and a re-evaluation of the role of the academic library. From the concept of the journal we are moving to single articles, and from libraries we are moving to end-user access.

## Confusion

There is a danger, therefore, of the whole area of networking becoming a confused mass of inconsistent applications. Global coordination is clearly required if we are to escape the present chaos and fashion our networks into a true information infrastructure. Networks must be made more flexible so that they can transmit information with the speed, security and reliability appropriate for different tasks; they must offer common services, such as directories of users and services; and they must share common communications conventions.

Despite the development, testing and management already under way, the largest single barrier to achieving these capabilities is the absence of high-speed networks. What is needed is a commitment to build fibre-optic systems and to improve existing copper-wire telecommunications, and this will certainly require considerable investment and expenditure.

But typically, policy lags behind both software and hardware development. Success will ultimately depend on detailed planning similar to that presented in the papers on the National Research and Education Network produced for the US Senate. The lessons from Japan, where electronic publishing and networking have already played a large part in the growth of science, show the urgent need for a national focus that will build public support and influence public policy.

Clarity of requirement is too often lacking in the schemes of enthusiasts, but without clear and detailed planning — and substantial investment — the full joint benefits to scientists of networks and electronic publishing will never be reaped. □

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## New Journals review 1992

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:
(1) the first number appeared during or after June 1990 and at least four separate numbers were issued by the end of April 1992 (although some of the journals not covered in last year's review issue were also considered)*;
(2) the journal is published at least three times a year;
(3) the main language used is English;
(4) where possible, at least four issues should be made available for review, including the first and the most recent numbers.

The time criteria ensure that a reasonable sample of issues is available for judgement by the time reviews are commissioned in June.

Several journals known to satisfy the above criteria were not submitted for review, or arrived too late for inclusion. It proved difficult to find reviewers for other, doubtless worthy journals, while some titles were considered to be of marginal interest to *Nature*'s audience. Journals covering any aspect of science were eligible, although those dealing with clinical medicine, engineering and pure mathematics were excluded, as were abstracts publications and newsletters. A list of titles eligible for review but not covered appears on page 464.

The brief given to the reviewers was to limit themselves to comments on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1992 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1992. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rate with the publisher concerned. □

* See *Nature* **353**, 457–481 (1991); **347**, 581–599 (1990); **341**, 350–370 (1989); **335**, 459–478 (1988); **329**, 357–376 (1987); **323**, 359–379 (1986); **317**, 293–308 (1985); **311**, 309–330 (1984); **305**, 477–497 (1983); and **299**, 491–514 (1982).

# Bringing it all back home

*James Lovelock*

**Science Probe! The Amateur Scientist's Journal.** Editor Forrest M. Mims III. *Science Probe Inc., 500–B Bi-County Boulevard, Farmingdale, New York 11735, USA. 12/yr. US $11.95, Canada $16.95, elsewhere $19.45.*

DID you see those violet coloured skies that followed sunset after the volcano El Chichón erupted in the early eighties, the visible expression of a stratospheric aerosol of volcanic sulphuric acid? Last year, the volcano Pinatubo injected an even larger mass of sulphur into the stratosphere but I have looked in vain for colourful night skies. It seems odd that millions of tons of sulphur dioxide injected into the stratosphere by one volcano in the northern tropics make colours visible in the sky in western Europe, but an even larger quantity from another volcano does not. I find it odder still that neither the journals of science nor the news media have commented on the colours of the night sky since the eruption of Pinatubo. Perhaps they think sunsets are just for poets.

Royal Society

**Red skies over Chelsea Harbour, London, following the eruption of Krakatoa in 1883. This crayon sketch is one of a series made shortly after sunset on 26 November 1883 by W. Ascroft.**

Pinatubo and the night sky were covered in the new magazine *Science Probe!*. An informative article answered many of my questions, and even showed how to estimate the height of the aerosol layer from the length of time colours lingered after sunset. This journal brings back a world of which science was a familiar part. For me it recalled an altogether lighter and more friendly *Scientific American*, read with joy in the public library at Brixton, south London, 60 years ago. Such reading and amateur experiments led me to a fulfilling life in the vocation of science, reading that was the antidote to the scientifically correct but utterly dull teaching of my grammar school. Science taught then, as now, was mere knowledge needed to pass exams.

*Science Probe!* is a cornucopia of delights for the amateur scientist and, I suspect, of real value also to the professional. It bears such treasures as the telephone number through which to obtain graphic images, in a format compatible with your personal computer, from the Hubble telescope; how to make an electrocardiogram; and how to encounter slime moulds in their natural habitat. It is transdisciplinary and regards all science as open to the amateur. In her clear and concisely written article, Valerie Villarreal, a high-school student, describes her project, "The Role of Capsaicin in Carcinogenesis". In another article, Mark Hartwig makes basic statistics so lucid that some professional scientists who read it may be able to distinguish precision from accuracy.

Science is criticized by some philosophers as soulless and damaging. This may be true of that part of science which has become too serious, narrowly specialized and subject to the strictures of scientific correctness; or of competitive fields, such as AIDS research, that are flawed, like the Olympics, by hubris. Science has lost soul; who would want to do chemistry for fun in the all-too-safe laboratory today? How did we allow dogma to become respectable and speculation pejorative? I grew up in science thinking that our task was to reduce science fiction to practice and have done my best to do so. I hope that *Science Probe!* flourishes and brings back science as something interesting that can be done at home. □

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# Mentioned in dispatches

*Sydney Brenner*

**Current Biology.** Editor Peter Newmark. *Current Biology. 12/yr. £180, $300 (institutional); £60, $99 (personal); £29.95, $49.95 (student).*

THE first issue of this journal appeared in February 1991, and although the journal is still fairly new, it has already made its mark, if only with its attractive and colourful covers. *Current Biology* aims "to inform its readers of outstanding developments in all areas of 'modern' biology"; each article is written by an expert in the field. I noticed that several of the articles had more than one author — one as many as four — which suggests that, in the minds of some of the authors, the article may not simply be a review of the outstanding development but that it may have developed something outstanding itself; and, as in all other scientific work, there seems to be a requirement that this be communalized to make sure that both honour and blame are correctly apportioned. The accepted way of both doing scientific work and writing about it has forced a rigid conventionality on us, and has stifled the individual. However, I am happy to report that the corporate contributions are very much in the minority. Most of the articles are extremely well written with an individual style and are what the cover claims — dispatches from the front lines of biology. I suspect that there is some considerable activity in the editing department, but, if so, it has been subtly applied and the articles do not read as though they had all been squeezed out of the same tube.

By far the most distinctive feature of the journal is the illustrations. Colour abounds, not only on the covers but also in photographs and in the special illustrative diagrams and cartoons provided for the articles. Some issues also contain a section called "Biology in Pictures". For example, in the March 1992 issue there is a display of ethereal panels of zebrafish development and another of striking photographs of genetic disease in mice and men. I do not like the portrait of Fred Sanger featured in the May 1992 issue; he looks too posed and fixed, as though cast in concrete, and he would normally not be looking at a gel with such vacant intensity, but instead would have been very pleased with what he was seeing.

*Current Biology* comes from the publishers of the *Current Opinion* series, which carry more organized and specialized reviews. The authors of these reviews choose and annotate important papers, and a selection of these references appears in *Current Biology*. This digest of digests is useful although, like some distillates, it can be quite heady; but perhaps some of the articles will drive readers back to the original papers for a longer drink.

The reviews cover all areas of biology. The very first has the title "DNA recombination in the brain?" — was this perhaps an unconscious hint of what the journal was aiming to do but with different technology? There is a very good article on "Miocene DNA sequences — a dream come true?", another archive we are trying to read. Everything is covered and even if there are reviews of similar fields in other journals, it does not matter, because the pace of advance is so rapid that we need such reviews in regular doses. An example is the field of cell signalling, and especially the G and GAP proteins, which need continuous treatment. As in so many other fields of research, an arcane tribal language has developed and the primary papers have become totally indigestible.

When I first started doing science, and even later in the 1950s in the early days of molecular biology, there was no rush about acquiring information. There wasn't very much of it about anyway, and a few months here or there made little difference. Perusing the *Annual Reviews of Biochemistry* sufficed to keep one up to date, and some of us actually read books to learn about other fields. We also had friends that we talked to about science, and scientific news also travelled in letters and preprints. But everything was doomed to success; the field deepened and widened, journals and meetings multiplied, and the whole impossible managerial structure of science as we know it today came into being. Francis Crick once told me that he had stopped reading the literature in molecular biology because he was sure that if anything important happened, somebody would tell him about it. The trouble is that today he would have an endless queue outside his door and would listen to nothing else. Most scientists have survived because they live in what I once called 'reading communes'. Fortunately, graduate students are compelled to read the primary literature and so, in the large laboratories, with their journal clubs, seminars and visiting speakers, it is possible to keep up. Those who do not enjoy (or suffer) that privilege need something like *Current Biology*. I only

## Journals index

hope that the editor can maintain both the stream of articles and the high scientific standards achieved, and that the publisher can continue to provide a most attractive and technically superior product without going bankrupt. □

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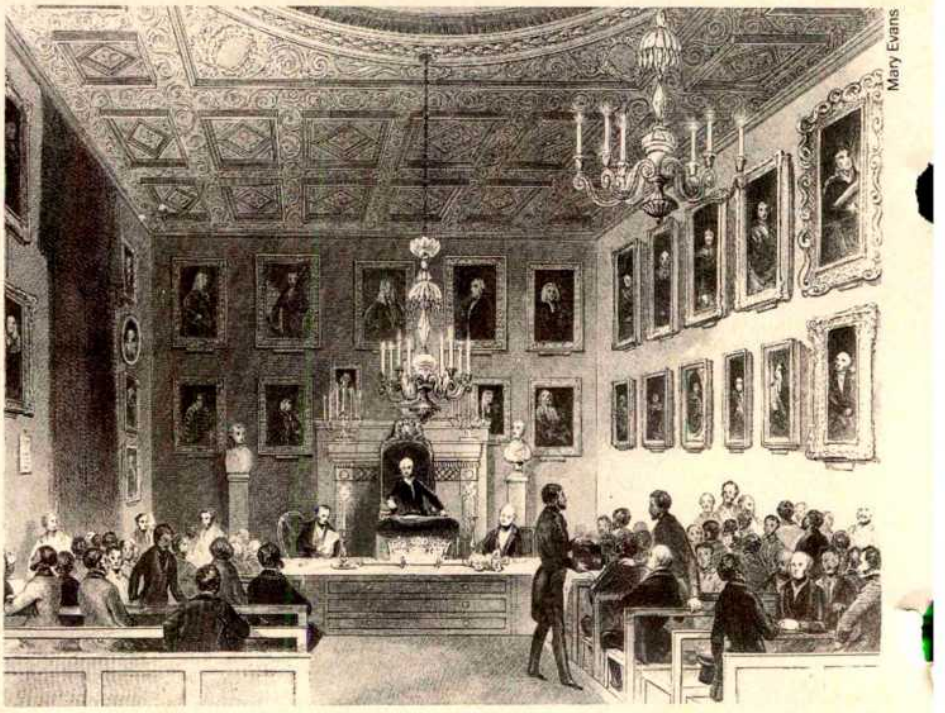
# Old wine in new bottles

*Christopher Wills*

**Philosophical Transactions of the Royal Society: Biological Sciences (Series B).** Editor Quentin Bone. *Royal Society. 12/yr. UK £525; elsewhere £560, $1,120.*

**Proceedings of the Royal Society: Biological Sciences (Series B).** Editor Bryan C. Clarke. *Royal Society. 12/yr. UK £200; elsewhere £213, $426.*



Mary Evans

**An early meeting of the Royal Society, drawn by Fairholt in 1840.**

A PHYSIOLOGIST friend of mine gave blood recently, a couple of hours after eating a hamburger with a side order of chips (french fries on my side of the Atlantic). Glancing with the usual curiosity at the transparent bag containing his donation, he was horrified to see that there was a creamy layer of fat globules at the top, like the head on a tankard of Guinness. Although he knew in theory that this should happen, he told me that the shock was considerable, and quite enough to put him off junk food permanently.

I thought of his tale while browsing through some early issues of the *Philosophical Transactions of the Royal Society*. In the sixth number, soon after the *Transactions* began publishing in 1665, there was a communication from Robert Boyle (of Boyle's law). He had observed a similar creamy layer in the blood of a housemaid who had eaten a hearty breakfast and shortly afterwards been bled by a doctor. To Boyle and his contemporaries, fascinated by everything they saw during that bright morning of the scientific method, this was nothing more than a puzzling observation. More than three centuries later, we know better. The dread of fat in the diet has now led many Americans to begin their day by eating the indigestible parts of plants (although the English breakfast still tends to be as fatty as that seventeenth-century housemaid's must have been).

One thing has not altered in the more than 300 years since the Royal Society's venerable journal first appeared, and that is the human species' endless curiosity about how the world works. This curiosity has changed our lives totally, not just our diets. It is still occasionally glimpsed even in the dense thickets of scientific prose that are found in the highly technical journals that glut our college libraries, shyly lurking behind the drab olive foliage of the passive voice. And it is very pleasant to find it alive and well in the *Biological Sciences* sections of the *Philosophical Transactions of the Royal Society* and its younger companion, the *Proceedings*.

This pair of distinguished journals has during the past two years undergone an editorial revamping, enough to justify their appearance in this supplement. Before this transformation, the *Transactions*, though it still covered a great variety of subjects, had tended to devote most issues to one or two large monographs. Now, says editor Quentin Bone, fewer such magisterial efforts will be published and they will be limited to the still ample length of 25,000 words (most of the papers in recent issues have been much shorter than that). The *Proceedings*, under the stewardship of Bryan Clarke, is emphasizing the rapid publication of very short papers. The editor's instructions to authors for both journals used to begin with the delphic utterance: "The Royal Society welcomes suitable communications for publication in its scientific journals." This gave the impression that the society knew, through some ineffable procedure, what was o was not suitable. It is a relief to see that both journals now state clearly that they will consider papers on any aspect of biological science.

The range of papers is indeed considerable. In recent issues, the *Proceedings* has published papers on the evolution of the genetic code, PET scans of the frontal lobes during willed and routine activity, and the effects of dehorning African rhinos on levels of poaching. The *Transactions* has published papers on the articulation of trilobites, the spread of the human immunodeficiency virus in New York City, and the best way to perform long jumps and high jumps. The periwigged contemporaries of Robert Boyle would, I think, have been delighted by this eclecticism.

The *Transactions* still devotes special issues to symposia covering single topics, but in between it ranges very widely indeed. In this era of the proliferation o highly specialized journals, the questio becomes whether there is still a place fo

■ General comments in the above review might equally well apply to the newly launched Series A journals of the Royal Society, *Philosophical Transactions: Physical Sciences and Engineering* and *Proceedings: Mathematical and Physical Sciences*. The first, edited by F. T. Smith, is published monthly and contains original papers, "Theme" issues and papers form the Society's discussion meetings (US and Canada £499, $998; elsewhere £466). The second, edited by J. E. Enderby and also published monthly, contains brief accounts of completed work that are normally published within three months of receipt, and longer papers (up to 15,000 words) normally published within seven months (US and Canada £384, $768; elsewhere £360).

publications such as these. I think there is, for two reasons.

First, in this age of computerized databases, none of these papers will go unnoticed by those who need to read them. Although none of the papers I read in the issues since the changeover were at the cutting-edge, they were all interesting and worthwhile.

Second, while these papers could undoubtedly all have found a home in specialized journals, the research library would be a much duller place as a result. Students would be denied the chance to stumble on journals that reflect the exuberance of the biological sciences as a whole. In short, if the *Transactions* and *Proceedings* save just a few budding biologists from joining the glassy-eyed ranks of the specialists, they will have served their purpose.

The revamping of these two journals has, I think, been successful. It has provided us with a stronger link to the time of science's bright morning. □

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# Botanical renaissance rag

*Philip Rubery*

**The Plant Journal.** Editors D. Bowles, M. Caboche, L. Dennis, D. Flavell, A. Komamine, J. Schell, C. Somerville and L. Willmitzer. *Blackwell Scientific. 6/yr (12/yr in 1993). 1993 prices: UK £340, US and Canada $675, elsewhere £374 (institutional); UK £85, US and Canada $165, elsewhere £91 (personal). (Members' rates on application.)*

THIS journal has the subtitle banner "For Cell and Molecular Biology" surmounting the photograph that dominates the front cover. The soil from which the green shoots of the journal emerged was the opportunity to approach plant biology 'in the round' — for the first time, according to the editors' preface to the inaugural issue in January 1991. Although the shades of Aristotle and Theophrastus might enjoy disputing this assertion, the contemporary point is that the sum of molecular and so-called 'classical' approaches exceeds their individual parts and is engendering a botanical renaissance. The editor's list of welcome disciplines runs the gamut from biochemistry to genetics, concluding "and, indeed, botany". It is a shame that botany has become almost a pejorative term; 'Plant Science' looks better on the departmental writing paper, and newspeak gets grants. 'Botany' derives from a Greek verb for pasture grazing (βόσκειν), whereas the practical Romans used the word for the sole of the foot (*planta*) to describe the shoot that it thrusts into the earth. (Which offers the more apposite metaphor for scientific funding practices?)

The broad range and high quality of the journal's contents so far justifies a quantitative measure of its success in moving from six to twelve issues per year in 1993. Its closest competitor is *The Plant Cell*, the stablemate of *Plant Physiology*, both owned by the American Society of Plant Physiologists (whose membership is debating a name change). Although there is interpenetration, authors' affiliations are predominantly North American in 'The Cell' and European in 'The Journal'. The majority of the papers concern genes one way or another, frequently making use of transgenic plants to do experiments that would have seemed fantastic only a few years ago. Two examples must suffice. From the first issue: the targeted expression of yeast invertase in vacuoles, cytoplasm and cell walls of tobacco plants is used to investigate carbohydrate metabolism at the whole plant level. From the latest issue to hand: the differential suppression of carotenoid levels in tomato fruit (but not leaves) in plants that express antisense RNA to a gene involved in the later stages of phytoene biosynthesis. Important papers in other, less immediately genetic, fields include molecularly informed work on cell signalling and cell lineage.

*The Plant Journal* also carries "Technical Advance" reports and fairly substantial "Mini-Reviews". One in particular, by Jeffery Dangl, is a stimulating discussion of possible parallels between the genetic basis of plant disease resistance and the major histocompatibility complex of the mammalian immune system. The necessary explanation of the intricacies of antigen presentation and recognition is rendered more palatable than usual by an extended 'restaurant' metaphor which, after a brief period of digestion, left one hungry for more.

This journal is good value for time and money (especially to members of the Society for Experimental Biology, who get a discount) and can be recommended to individuals and to librarians. □

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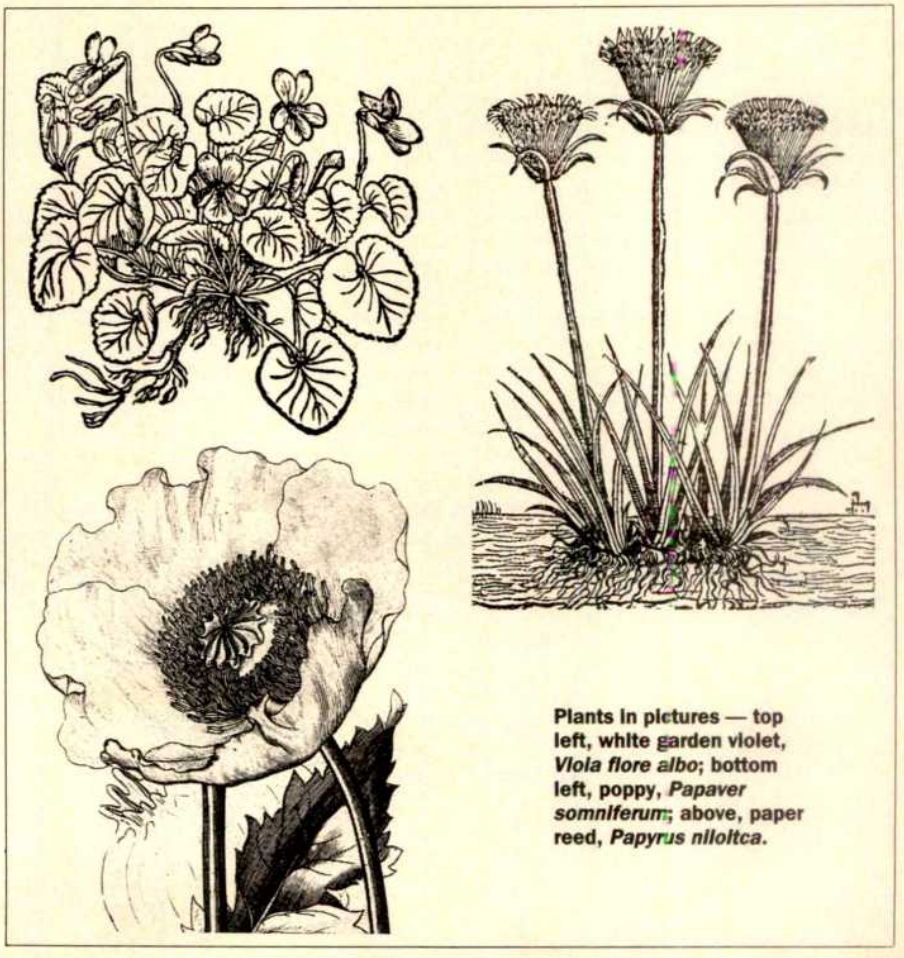

**Plants in pictures — top left, white garden violet, *Viola flore albo*; bottom left, poppy, *Papaver somniferum*; above, paper reed, *Papyrus niloitca*.**

# Project reading

*Neal G. Copeland*

**Mammalian Genome: Official Journal of the International Mammalian Genome Society.** Editors Lee M. Silver, Joe Nadeau and Jan Klein. *Springer. 12/yr. $269 (institutional), $129 (personal).*

NOT only has the Human Genome Project led to a revolution in the pace of genome research, it has also spawned several new dedicated journals. In its original conception, *Mammalian Genome* was to provide a voice for the International Mammalian Genome Society and an archive of information related to the mouse genome project. In its final conception, the goals of the journal are much broader and include the publication of new findings in all areas of mammalian genome research, including those pertaining to humans. In scope, therefore, the journal overlaps considerably with other highly successful genome journals such as *Genomics* and *Nature Genetics*. The long-term success of *Mammalian Genome* will no doubt therefore be determined by how well it competes with other genome-related journals for publication of the most exciting research findings in the field.

The first volume was published in 1991 and consisted of four issues. In 1992, 12 issues are to be published, attesting to the early success of the journal. Each issue contains a number of original papers published as full-length articles, brief communications or brief reports of data, in addition to an occasional review. The reviews are generally good, dealing with such diverse topics as obesity, mapping with mononucleotide repeats, dispersed repetitive elements in mouse genome analysis, positional cloning and mouse pigment mutations.

The editorial board is excellent and is composed of 42 leading scientists actively engaged in all facets of mammalian genome research (although heavily skewed towards mouse research). Most papers published so far have involved the mouse, although a smattering of papers dealing with the genetics of humans and other model organisms (rat, mink, fox and cow) have also appeared. Much of what has been published is straightforward linkage analysis. The scope of the papers is often somewhat narrow; but nonetheless they are important contributions to the field. I expect that as the journal matures and is even more widely read, the scope and quality of the papers will improve accordingly.

A subscription to *Mammalian Genome* includes membership of the International Mammalian Genome Society and receipt of a special supplementary issue, compiled yearly, containing committee reports for each of the mouse chromosomes. The reports provide the most complete and up-to-date linkage information available for the mouse and are a must for anyone engaged in or contemplating work on the mouse. The special issue alone is worth the cost of a yearly personal subscription. My only complaint against the reports is that they do not follow a single format. Each chromosome committee is left to its own devices to decide which data to include, which to leave out and in which format the final report should be written. This often makes it difficult for all but the most ardent mouse *aficionado* to extract information.

As an official voice for the International Mammalian Genome Society, *Mammalian Genome* has been somewhat mute. Society news so far has consisted of a few meeting announcements and a report on the Fourth International Mouse Mapping Workshop. Perhaps such news will increase as membership of the society grows and the society begins to play a more active role in coordinating and orchestrating mammalian genome work.

In summary, *Mammalian Genome* is a good addition to the list of new journals spawned by the Human Genome Project. It will certainly be required reading for anyone working on the project and in particular those working in the field of model organisms. □

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# Limits of the growth business?

*Peter Little*

**Current Opinion in Genetics and Development.** General editors Ron Laskey and Matthew P. Scott. *Current Biology. 6/yr. £209.95, $390 (institutional); £69.95, $129.95 (personal); £29.95, $49.95 (student).*

REVIEWS are *the* publishing growth area in biological sciences and *Current Opinions in Genetics and Development* is one of the more interesting new arrivals in a crowded area. Genetics and development could encompass virtually the whole field of biology but there is massive competition. Realizing this, the publishers have used two unusual tools to make all *Current Opinion* journals different from their competitors, such as *Annual Reviews of Genetics*, or from review articles in journals such as *Cell* or *Development*. Each issue contains 15–20 articles constructed around a subject area such as gene organization and evolution, genetics of disease, gene expression and differentiation or pattern formation and development. The subject areas are always topical and the contributions, of between five and six pages, are almost uniformly excellent, well focused and concise.

What is, I think, unique is that authors are asked to annotate their reference lists, giving a brief précis and also indicating the papers in their field that they see as being of "interest" or "outstanding interest". This is a clever idea: it allows the (relative) outsider to assess not only the main facts of a field, as does any review format, but also its intellectual and social structure. The reviews detail the important observations and models, and the annotations place them in context. By design this scheme explicitly gives a personal opinion as to who is doing important work, which is otherwise only discernible by careful filtering of more extensive reference lists from conventional reviews. I am not sure that this procedure will appeal to everyone: the papers are perhaps too selective for the specialist. But for scientists who wish to explore the peripheries of their field, they are ideal.

The second unique feature of all the *Current Opinion* series is that they include a large bibliography of current literature relevant to the field under review. This is extensive, covering more than 40 pages divided into three columns. The full authorship and title of each paper are listed but there are no abstracts. I am not convinced that this bibliography is useful, because it is essentially a paper listing of the computerized literature searches that I routinely use, but with none of the specific filters included to make the list manageable. I have never felt the need to use these lists, but perhaps that is a personal failing.

I have found *Current Opinion in Genetics and Development* particularly useful in approaching new areas of my own research, which for me is a certain measure of a review's success. This is a continually interesting journal and moderately priced; it should be part of any well equipped personal or institutional library. □

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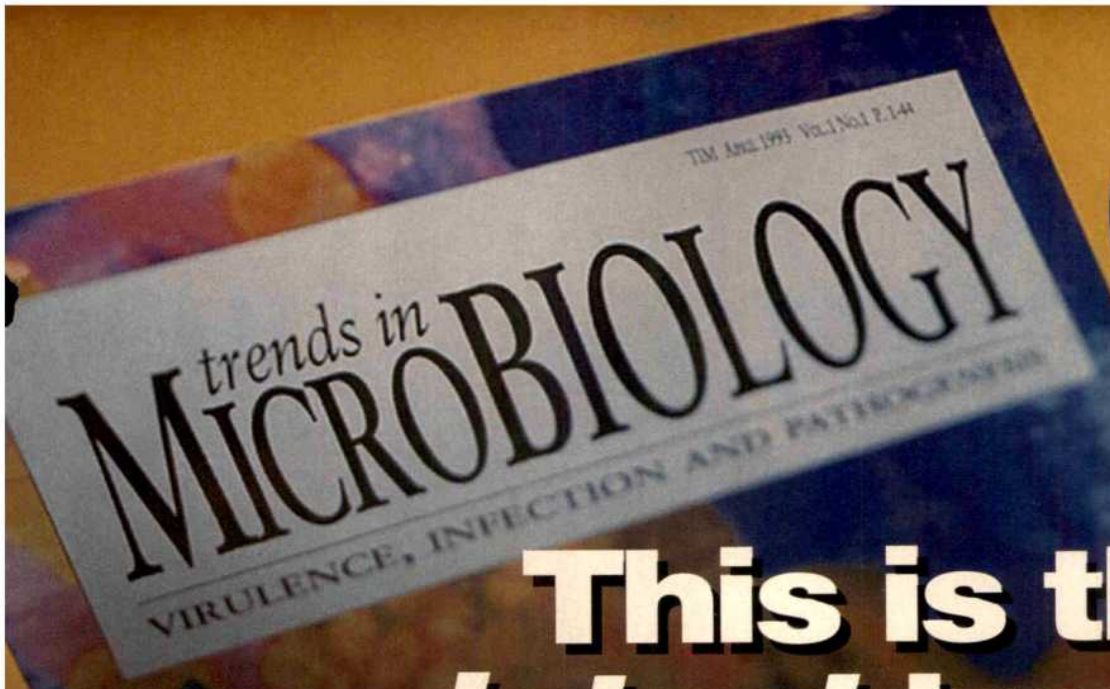


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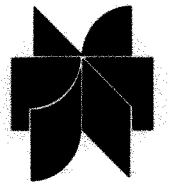

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# Cellular communication

*John Armstrong*

**Trends in Cell Biology.** Editor Carol Featherstone. *Elsevier. 12/yr. All countries £239 (institutional); US and Canada £92, UK £59, elsewhere £63 (personal).*

IF any readers still need to be convinced that cell biology is a trendy subject, this latest addition to Elsevier's stable of popular review journals should do the trick. *Trends in Cell Biology* follows the general format established by *Trends in Biochemical Sciences* (*TIBS*), offering a monthly collection of reviews of hot topics and developments, all of readable length, embellished not only with figures but also with the distinctively baroque scientific cartoons pioneered by *TIBS*. From the first issue in July 1991, the aim of conveying the excitement of the field has been apparent, with the promise of centrefold spreads and even a "Forum" section. Only the small print on the last page appeared to dampen the atmosphere, with its statement that the journal is "apolitical", threatening to exclude many distinguished potential contributors. Happily, this does not seem to have been the case.

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**False colour SEM of hair cells (yellow) in the organ of Corti in the inner ear (× 6,747).**

Review journals have a range of target audiences, among them advanced students, hard-pressed teachers and researchers. The *Trends* journals aim to cater for all these groups, and in its first year *Trends in Cell Biology* has done well in achieving its goals. Perhaps the hardest task in editing a review journal is finding the right contributors, given that cell biology is no better endowed than any other scientific discipline with practitioners who view the written word as a means of communication rather than an obstacle to it. In some of the early issues there are hints of too gentle an editorial hand: who needs "Experiments have shown that", or wet-blanket conclusions such as "The combination of . . . techniques should continue to provide new insights into the molecular basis of . . ."? Fortunately, the journal's confidence seems to be growing; as well as attracting good authors, perhaps it may embolden others to write imaginatively. In this respect the "Headlines" feature can only be a good thing: single-paragraph summaries of particularly important papers are individually contributed by cell biologists from the younger end of the age spectrum, but authors are credited as a group.

Several other review journals have appeared in recent years, but, curiously, the biggest competition to *Trends in Cell Biology* might be from within. It is not hard to think of articles that could fit into this journal, *TIBS*, *Trends in Genetics* or *Immunology Today*, for example, and the publishers may eventually decide that not every field remains equally trendy. It must surely be in the interests of cell biologists to ensure that their subject is more equal than the rest. □

*John Armstrong is in the School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK.*

# Identity crisis

*P. R. Stanfield*

**Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry and Pharmacology.** Managing editor F. Lang. *Karger. 6/yr. SFr334, $223, £145 (institutional); SFr223.80, $156.10, £101.50 (personal).*

ALTHOUGH physiology is classically an integrative discipline, many of its current success stories are in work at the cellular or molecular level, at the interface with molecular biology, biochemistry and biochemical pharmacology. Work on ion channels, with single-channel recording and other molecular approaches, is an obvious example. A journal dedicated to this interface and committed to publishing original papers of high scientific quality "pertinent to cellular function and its regulation" should, then, be a real success story. This might be expected all the more when the journal has an editorial board that varies from the merely excellent to the Nobel prizewinning. Yet *Cellular Physiology and Biochemistry* has not really established itself at the forefront, let alone entered the consciousness of many scientists at all.

This is not to say that the papers published have not been good; often they have come from outstanding laboratories. The journal also publishes useful brief reviews, and one issue (combining numbers 3 and 4 of volume 2) was composed of a series of reviews on ion transport in the regulation of cell proliferation and published in memory of the late Ephraim Racker, one of the journal's most distinguished original editors.

The quality of publishing is also high, with double-column format, clear printing free from typographical error, elegant production of diagrams, often in an outlining box on a grey background, and excellent colour printing within the text of papers.

With so much going for it, why is the journal not better known? Part of the reason may lie in simple technicalities, such as not yet appearing in *Current Contents*. Part may be the expense at a time when library budgets are being squeezed. But perhaps in generously drawing wide boundaries in defining its scope — publishing cellular work irrespective of "the questions asked, the methods applied or the tissues analysed" — the journal has failed to find a clear identity. □

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# Exponential growth

*Herb F. Sewell*

**European Cytokine Network.** Editors-in-chief Jacques Bertoglio and Didier Fradelizi. *John Libbey Eurotext, 6 rue Blance, 92120 Montrouge, France. 6/yr. France FF1,100, rest of Europe $195, elsewhere $195 (institutional); France FF850, rest of Europe $135, elsewhere $150 (personal); France FF500, rest of Europe $90, elsewhere $105 (student).*

DURING the past decade, the growth of data in publications covering the biological, biochemical, biophysical and clinical applications of cytokines has been strikingly exponential. It has been nearly impossible for even the most dedicated basic researchers, users of cytokines and clinicians to keep up with all the latest developments. What they required was a publication of original, comprehensive, concise, informative and up-to-date articles. These requirements have now been ably met by the *European Cytokine Network* journal. The editors' intention of providing the community with a publication of interest to a large audience has been excellently maintained; this journal 'networks' scientists and practitioners in immunology, molecular biology, oncology, physiology — indeed in most of the recognized 'ologies' — informing them of the latest developments in the unifying subdiscipline of 'cytokine-ology'.

The members of the distinguished editorial board have worked hard and stuck to their brief: original articles reflect state-of-the-art science and comprehensively cover most cytokines without the usual overemphasis on the prototypic molecules such as the interleukins 1 and 2 and granulocyte–macrophage colony-stimulating factor. It is noteworthy that for original articles there is a gap of four to six weeks between reception and acceptance, with publication usually within eight to ten weeks.

Another attractive aspect of the journal is the comprehensive scientific reviews and mini-reviews, some six or four pages of highly informative, well referenced and thought-provoking commentary by leading experts. There are also occasional detailed technical papers, crucial for evaluating the science in this burgeoning field. The journal's continued good performance and quality is in part due to the welcome practice of commissioning notable annual guest editors. The quality of the illustrations and the print has undergone considerable improvement, and the journal's annual subscription rates compare very favourably with journals in other specialist disciplines.

Although we will all continue to browse in our libraries, chase our CD-ROMs and listen attentively at 'update meetings', there is no doubt that this journal represents one of the best attempts at networking the discipline and the disciples of cytokines. If one is interested in bridging the gap from cytokine biophysics, to biology, to bedside, then this journal is a must. Its genesis was most timely, and its infancy and formative first two years indicate that it will continue to nurture and network its readers. □

*Herb F. Sewell is in the Department of Immunology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, UK.*

# Mass media

*J. K. Heath*

**Growth Regulation.** Editor D. Schulster. *Churchill Livingstone. 4/yr. $240, £137 (institutional); $191, £109 (personal).*

THE origins of research on growth factors lie in many fields, but a major contribution came from developments in endocrinology. Nowadays 'growth factors' are perhaps more associated in the minds of readers of *Nature* with cell or developmental biology, although there has always been a steady undercurrent of important growth-factor work published in the mainstream endocrinology journals. This has been especially true for work on circulating growth factors such as growth hormone and the insulin-like growth factors (IGFs or somatomedins). *Growth Regulation* is aimed at capturing this segment of the market. Indeed, growth — as in size — is exactly what the journal is all about and most of the papers are devoted to studies of growth factors that control body mass.

In *Growth Regulation* one is, therefore, more likely to read about RIA and IGF than RNA and FGF. Most papers in the first few issues are devoted to various aspects of the IGFs, including studies in both cell culture and human subjects. So if the journal is going to be a long-term success, it will probably be as the somatomedin-fraternity house journal rather than as a direct threat to the main established endocrinology journals. It certainly remains to be seen whether it can spread from this niche and truly reflect the interdisciplinary approach to growth regulation predicted in its opening leading article.

The editors do, in fact, have something of a dilemma on their hands. Given the interest in the subject from outside the scientific community, the control of body mass is bound to be a problem that attracts increasing attention, some of which is likely to be anecdotal rather than scientifically rigorous. This is illustrated by one of the weakest papers in the opening issues, a study of IGF levels in prepubertal female gymnasts undergoing intensive training. I was caught between wanting a better piece of science and the potential implications, which otherwise might as well be summarized as 'Don't put your daughter on the beam Mrs Worthington'. Despite these misgivings, the general quality of many of the contributed papers in the early issues is reasonable. The journal also includes review articles and a useful literature survey. Like most new journals, *Growth Regulation* has staked out a niche in the market and will succeed if the niche itself continues to expand. □

*J. K. Heath is in the Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.*

# Cancer forum

*David A. Cheresh*

**Melanoma Research.** Editors Ferdy Lejeune, Guiseppe Prota and Patrick Riley. *Rapid Communications of Oxford. 6/yr. £180, $342.*

HUMAN melanoma is widely studied by basic and clinical researchers for several reasons. First, it is among the most malignant cancers known. Second, the metastatic form of this tumour does not respond to treatment. Third, the incidence of melanoma is on the rise. Fourth, there are many cultured melanoma cell lines and animal models for investigation.

So far, many of the published articles on human melanoma have appeared either in strictly basic science or clinical journals. *Melanoma Research* aims to provide a new international forum for the rapid dissemination of basic and clinical melanoma research. It presents a broad scope of basic articles on biological studies with emphasis on genetic, molecular or biochemical findings. The clinical articles focus on diagnostic approaches and novel therapies and their effectiveness in clinical trials.

The standard of the articles is comparable to that of more well established cancer journals. Each issue contains relevant reviews and a good mixture of basic and clinically related articles. The production quality and format seem

adequate and the average number of articles per issue over the first year was seven. In that year, 72 manuscripts were received, 25 of which were rejected outright; most of the accepted manuscripts required revision. So it seems that the editorial board is attempting to promote a good-quality yet focused journal. The fact that more than two-thirds of the papers were reviewed within two weeks will help to continue to attract research articles of a high standard. On the basis of its good start, and blend of basic and clinical articles, *Melanoma Research* has an excellent chance of survival. □

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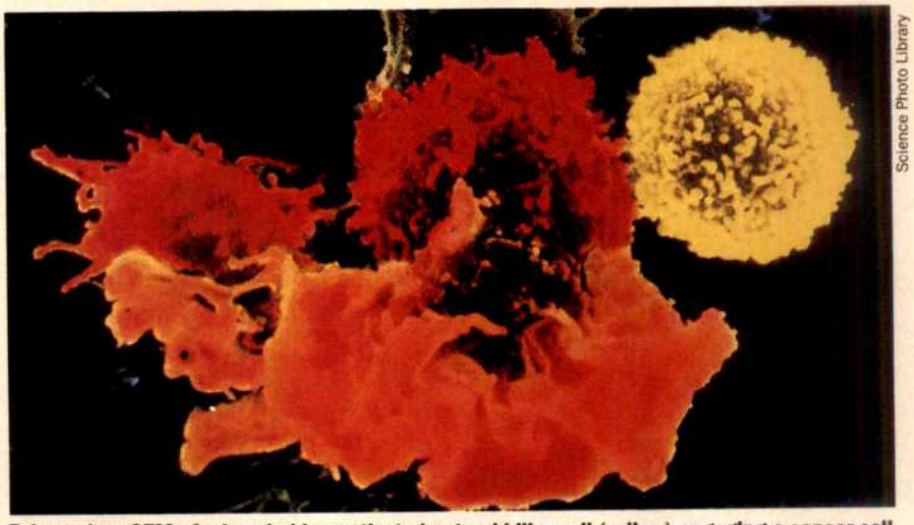

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**False colour SEM of a lymphokine-activated natural killer cell (yellow) engaging a cancer cell (red) (× 7,770).**

# Controversial issues

*L. J. Kinlen*

**European Journal of Cancer Prevention.** Editors M. J. Hill and A. Giacosa. *Rapid Communications of Oxford. 6/yr. £167, $299.*

THIS is the second journal on cancer epidemiology, launched only a year after *Cancer Causes and Control* from the same publisher (reviewed in last year's New Journals issue (*Nature* **353,** 467; 1991)). Its stated aims are to fill a gap in the periodical market on both the specialized subject of metabolic epidemiology and the broader field of cancer prevention, which has resulted in many relevant papers being "lost to the scientific world". Such a gap may exist for the first subject, but the second is covered by, for example, the publisher's other recent journal. The latest journal is also the organ of the European Cancer Prevention Organisation, which has organized several meetings over the past 12 years. It includes papers presented at some of these meetings and reports of studies sponsored by the organization (one having the engaging title "ECP-EURONUT-IM Study").

In the first four issues (up to June 1992), about two-thirds of papers concerned gastrointestinal cancers. But for other malignancies, a question of editorial balance arises — in the second issue, in an article entitled "Controversy", the proposition is set up for debate that work on the role of human viruses is "largely wasted" because there is "still little firm evidence for such a role". This is hardly a serious scientific proposition. Indeed, a feature of recent work in cancer aetiology is the mounting evidence for the role of viruses, the more striking given the disappointment of much 1970s work. Examples include HTLV-1 in T-cell leukaemia and human papilloma viruses in cervical cancer. Yet in this surprising article, such work is dismissed. Furthermore, in hepatocellular cancer (worldwide, one of the ten commonest cancers), the role of hepatitis B virus is played down because the author regards it as mediated by cirrhosis — as though alcohol could be said to have no role in road accidents because its primary effects are on human behaviour. The editors mention that no one could be found who was interested in responding.

Attractively produced, *European Journal of Cancer Prevention* reflects current interest in adenocarcinoma of the oesophagus as well as containing some intriguing speculation about the Mediterranean diet and its possible protective effects against cancer. The journal will be of interest to members of the European Cancer Prevention Organisation and to those concerned with the epidemiology of gastrointestinal cancer. But its value to others is less clear. □

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# Channels of reception

*Henry R. Bourne*

**Receptor.** Editor-in-chief Gerald Litwack. Humana. 4/yr. *$150.*

SOME of us can remember when receptors existed only as hypotheses, of interest to pharmacologists and a few endocrinologists. Now, hardly any biologist can enter the laboratory without stumbling over a receptor, whether the laboratory studies are on cells, genes, animals, plants, protein structure, development or neurobiology. In every field, receptors abound — hence this new journal.

According to an editorial note in its first issue (late 1990), *Receptor* seeks to publish papers in "the biochemistry, pharmacology, cell biology, microbiology, molecular biology, and biophysics of every type of receptor". How does this work out in practice? The 15 original reports in the three issues I surveyed described effects of receptor agonists on various events studied in tissue culture cells (seven papers), effects of heat, stress or hormones on receptor abundance in cells or animal tissues (three), biochemical characterization of receptors (three) and ligand-binding assays (two). Surprisingly, not one paper evinced more than a passing interest in drugs. In the same 15 papers, eight receptors regulate transcription (receptors for steroids and thyroxine), four are coupled to G proteins, two are receptor tyrosine kinases, one binds prolactin and none is (or regulates) an ion channel.

What about quality? In my opinion, the research reports earn a rating of 'good, but not excellent'. Most present sound, well-executed experiments dealing with more-or-less well understood phenomena, confirming and extending what we already know: solid, reliable papers, with no surprises. The reviews are thorough, well written and of interest to investigators of receptors and other signalling proteins. Like the reports, the reviews reflect on predominant interests of the editor and editorial board: of four reviews in the issues I sampled, three deal with steroid receptors, one

with receptors coupled to G proteins.

From the reader's point of view, *Receptor* has two irritating defects, both easy to fix. First, after listing the title and authors, each paper begins with a list of its contents, in outline form; this wasteful practice takes up space, tells us nothing we need to know and relegates the abstract of the paper onto a second page. Second, reviews are not identified as such in either the table of contents or elsewhere. □

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# Sugar daddy

*D. A. Rees*

**Glycobiology.** Editor-in-chief Gerald W. Hart. *Oxford University Press. 6/yr. Europe £150, elsewhere £250 (institutional); Europe £75, elsewhere $125 (personal).*

JOURNALS dedicated to carbohydrate technology, especially starch and cellulose, and to carbohydrate reviews, have been well established for many years. Specialist journals for fundamental carbohydrate research are of a more recent inception, and the launch of the first of these in the mid-1960s was not without controversy. Most of us then working on carbohydrate problems had spent our entire careers in the field and could trace scientific lineages through distinguished and similarly specialized mentors, all the way back to the beginnings of the subject. I was in the camp opposing the journal on the grounds that intellectual inbreeding had gone too far and was leading to a degree of isolation that might be exacerbated by separate research publications. Better, we believed, to break out to mix with other research areas than to keep to home territory. Some of us published and presented research on polysaccharide structure and function alongside similar work on proteins and nucleic acids, with much benefit to our methods and insights. Others contributed through their work on carbohydrate synthesis and reaction mechanisms to the development of central concepts of organic natural products as a whole. In the light of subsequent experience, the issues were not really as black and white as they seemed and I think the specialist journals have brought more good than bad. But still, a balance has to be kept and it is reasonable to ask again, as we did before, whether any new field-specific journal looks like a venture to establish a sanctuary to which it would be too easy for intellectual introverts to retreat.

Setting prejudice aside to read *Glycobiology* itself and to peruse the membership of the editorial board, it seems that history has by now brought about a strange inversion. It is refreshing that *Glycobiology* is engaging so many scientists who have entered from successful backgrounds in other fields or who continue to work in parallel in other fields. In his inaugural leading article, Gerald Hart argues the need for a forum that such scientists would otherwise lack. Instead of there being the danger of tunnel vision, this community now feels the need for a sharper focus. Instead of there being a tradition that might threaten to stifle, the new research constituency now needs more communication among its members.

I do not think it can be denied that the concept of a focal journal does have some problems. No single journal can publish more than a small proportion of the good work in such a sprawling field as glycobiology. The early issues of this new journal are handsomely produ and present scientific work of high q ity but, for example, the four represe tive issues I have been sent for rev contain very little indeed on plant prokaryotic systems. The main emph seems to be on mammalian glycoprot in relation to biosynthesis, transport recognition. Again, there seems to b hint that, like the quest for the H Grail, we need an enterprise to sea for a new biological function for car hydrates that has eluded us thus far this really a good bet?

Overall, the journal has excellent tures in the "Glyco-Forum", "M Review" and "Meeting Report" sectic These pull threads together with a car hydrate orientation not found anywh else in the scientific literature, and i for this reason that the journal deser to attract personal subscriptions. should also become a core item for serious library for biological research.

*D. A. Rees is at the Medical Resea Council, 20 Park Crescent, London W 4AL, UK.*

# Responses and reactions

*Ian R. Phillips*

**Pharmacogenetics.** Editors J. R. Idle, F. J. Gonzalez and R. Kato. *Chapman and Hall. 6/yr. US and Canada $263, Europe £142, elsewhere £152 (institutional); US and Canada $107, Europe £58, elsewhere £58 (personal).*

PHARMACOGENETICS, the study of genetically determined variation in response to drugs, was established more than 30 years ago. The field was later extended, with the coining of the term 'ecogenetics', to cover all environmental and xenobiotic agents.

As is pointed out in a commentary in the first issue of *Pharmacogenetics*, the discipline is founded on discoveries that differences in response to three important drugs in use at the time, namely the anti-malarial primaquine, the local anaesthetic dibucaine and the antitubercular isoniazid, were due, respectively, to inherited variations in glucose-6-phosphate dehydrogenase, pseudocholinesterase and acetyltransferase. It has since become clear that genetically determined variation in response to drugs is not confined to these classic textbook examples, but is an important factor underlying differential responses to many therapeutic drugs.

Pharmacogenetics, like many areas of biology, was revolutionized by the advent of molecular biology, which provided the probes and technology for determining the molecular genetics.

The journal publishes original search papers, leading articles, invi reviews and occasional invited comm taries, symposium proceedings and bc reviews. There is also scope for scient correspondence, although none appe in the early issues that I saw. The qua of production is excellent, as is speed publication, most papers appearing t to four months after submission, so even in the same month as their acce ance. In the more recent issues a balai is beginning to be achieved betwe papers on molecular aspects and those a more clinical or physiological natu But of the 24 research papers in the f five issues, 19 were from members of editorial board, and I detected a note desperation in the editorial pleas articles.

For me, the most successful feature the journal is the series of excell invited reviews, one in almost ev issue. Covering topics from across spectrum of pharmacogenetics, they a valuable contribution to the field.

*Pharmacogenetics* is a useful addit to the literature. But I suspect that succeed it will have to attract pap from a broader range of authors.

*Ian R. Phillips is in the Department Biochemistry, Queen Mary and Westfi College, University of London, Mile I Road, London E1 4NS, UK.*

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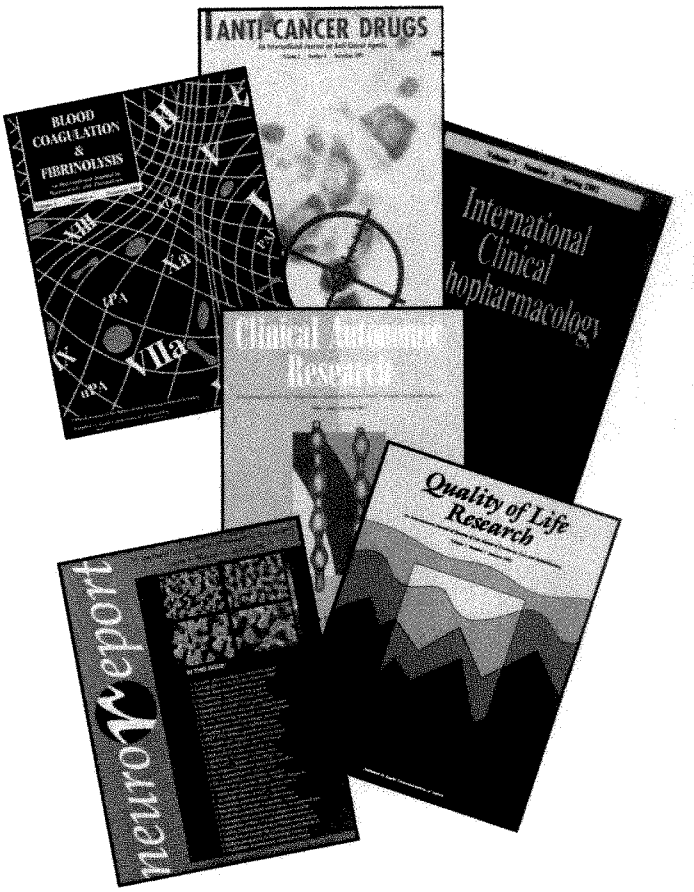


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# JOURNALS 1993

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## THERAPEUTIC IMMUNOLOGY

**Editors**
Ellen Vitetta *Cancer Immunobiology Center, University of Texas, Dallas, USA*
Herman Waldmann *Immunology Division, Addenbrooke's Hospital, Cambridge, UK*

Reporting from the frontline of immunological research. *Therapeutic Immunology* will publish the most up-to-the-minute applications of the scientific basis of immunology to the diagnosis and treatment of disease. The latest advances and developments will be reported in top quality original research papers; review articles written by experts will provide analysis; provocative editorial and opinion pieces will generate debate and discussion; and commissioned conference reports will inform on recent meetings.

*Therapeutic Immunology* will be published bimonthly, beginning in July 1993. ISSN 0967-0149 Volume 1, 1993, will comprise 3 issues; with Volume 2, 1994, comprising 6. Subscription prices for Volume 1, 1993 are as follows: *Individuals* £30.00 (Europe), £33.00 (Overseas), US$57.50 (USA) *Institutions* £60.00 (Europe), £66.00 (Overseas), US$115.00 (USA), *including postage.*

## MONTHLY IN 1993
## THE PLANT JOURNAL

**Editors**
Dianna Bowles, Michel Caboche, Liz Dennis, Dick Flavell, Atsushi Komamine, Jeff Schell, Chris Somerville, Lothar Willmitzer.

*The Plant Journal* is a broad-based high-quality journal of plant molecular sciences. Naturally this involves work pertaining to gene structure and function specifically, but also includes studies in biochemistry, cell biology, biotechnology, developmental biology, genetics and botany.

*The Plant Journal* is published monthly in 1993. ISSN 0960-7412. Subscription prices for Volume 3, 1993 are: *Institutional* £340.00 (Europe), £374.00 (Overseas), US$675.00 (USA) *Individual* £85.00 (Europe), £91.00 (Overseas), US$165.00 (USA) and the SEB members' rate is £60.00.

## INSECT MOLECULAR BIOLOGY

**Editors:**
Dr Julian Crampton, Dr Anthony A. James.

*Insect Molecular Biology* provides an international forum for all those applying molecular genetic techniques to the study of insects. Its prime purpose is the publication of original, high quality research papers on the structure, function, mapping, organization, expression and evolution of insect genomes.

*Insect Molecular Biology* is published quarterly. ISSN 0307-6975. Subscription prices for Volume 2, 1993 are: *Institutional,* £113.00 (Europe), £124.00 (Overseas), US$219.00 (USA); *Individual,* £56.50 (Europe), £62.00 (Overseas), US$109.00 (USA).

## NEW
## RESTORATION ECOLOGY

**Editor**
William A. Niering *Connecticut College, USA*
**Associate Editor** Edith B. Allen *San Diego State University, USA*

*Restoration Ecology* is a new, peer-reviewed quarterly journal published for the Society for Ecological Restoration. Edited by a distinguished panel, the journal will publish research papers, reviews, opinions of readers, and technical reports on the process of ecological restoration, defined as the intentional alteration of a site to establish a defined indigenous, historic ecosystem.

*Restoration Ecology* will be published quarterly. ISSN: 1061-2971. *Individuals:* US$65; Canada/Mexico: $70; Overseas: $85. *Institutions:* US$105; Canada/Mexico: $110; Overseas: $125. *Students:* US$25; Canada/Mexico: $30; Overseas: $45
The Society for Ecological Restoration offers special subscription rates to members. Please contact the Society for details.

## THE ISLAND ARC — NEW

**Editors-in-Chief**
Asahiko Taira *(Tokyo, Japan)* and Masayuki Komatsu *(Matsuyama, Japan)*
**Executive Editor** Kisaburo Kodama *(Tsukuba, Japan)*

*The Island Arc* is the official journal of the Geological Society of Japan in association with the Japan Association for Quarternary Research, the Japanese Association of Mineralogists, Petrologists and Economic Geologists, the Palaeontological Society of Japan and the Society of Resources Geology. The journal will focus mainly on the geological, geochemical and geophysical problems related to modern and ancient plate convergent processes.

*The Island Arc* is published quarterly. ISSN 1038-4871. The subscription rates for 1993 are Yen 32500 (Japan) and A$250.00/US$300.00/UK£115.00 (overseas). Subscribers will receive Volume 1, Issue 1 (August 1992) of *The Island Arc* FREE with their 1993 Subscription.

## 6 ISSUES in 1993
## MOLECULAR ECOLOGY

**Editors**
Terry Burke *University of Leicester, UK.* Ray Seidler *Corvallis, USA.* and Harry Smith *University of Leicester, UK*

Directed at the interface between molecular biology and the ecological sciences, *Molecular Ecology* publishes the results of research that uses molecular biological approaches to provide innovative insights into any aspect of ecology or population biology.

*Molecular Ecology* is published bimonthly. ISSN 0962-1083. Subscription prices for Volume 2, 1993 are: *Institutional,* £140.00 (Europe), £155.00 (Overseas), US$265.00 (USA); *Individual,* £30.00 (Europe), £35.00 (Overseas), US$57.00 (USA).

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***s: J R Idle, University of Newcastle upon Tyne, UK, F J Gonzalez, National ncer Institute, Bethesda, USA and R. Kato, Keio University, Tokyo, Japan***

ics covered by **Pharmacogenetics** include:

The identification and characterization of polymorphic genes, encoding drug-metabolizing enzymes, drug receptors and transporters and the effects of their expression on the disposition and metabolism of foreign chemicals, as well as the cellular and clinical responses they evoke.

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gulation of human alcohol dehydrogenase genes. *H J Edenberg and C J Brown, USA.*

novel bilirubin/phenol UDP-glucuronosyltransferase *UGT1* gene locus: implications for multiple hemolytic familial hyperbilirubinemia phenotypes. *I S Owens and J K Ritter, USA.*

*ginal Articles*

ermination of CYP1A2 and N-acetyltransferase 2 phenotypes in human populations by analysis of eine urinary metabolites. *M A Butler, et al,. USA and Italy.*

liver thiopurine methyltransferase pharmacogenetics: biochemical properties, liver-erythrocyte tion and presence of isozymes. *C L Szumlanski,et al. USA.*

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## Glycoconjugate Journal

Official Journal of the International Glycoconjugate Organization

***Chief Editor: Alan Chester, University Hospital, Lund, Sweden, Associate Editors: Kenneth Lloyd, Memorial Sloan-Kettering Cancer Center, New York, USA and Toshiaki Osawa, Yakult Central Institute, Tokyo, Japan. Special Advisory Editor: R. Dwek, University of Oxford, UK.***

The **Glycoconjugate Journal** is the established bimonthly journal publishing papers and reviews on all aspects of glycoconjugate research and glycobiology. The emphasis is on the metabolism and function of glycolipids, glycoproteins, oligo- and polysaccharides and proteoglycans. This includes the mechanisms of biosynthesis and biodegradation of glycoconjugates, their roles in health and disease and their interactions with other molecules. The Journal also publishes relevant information on the structure and synthesis of glycoconjugates and developments in methodology.

The **Glycoconjugate Journal** is the official journal of the International Glycoconjugate Organization, which organizes the biennial International Symposia on Glycoconjugates.

**It is covered by *Current Contents, Index Medicus* and *Excerpta Medica*.**

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Enzyme-linked immunosorbent assays for the measurement of blood group A and B glycosyltransferase activities. *L M Keshvara, et al., Canada.*

Surface glycoprotein of human natural killer cells recognized by wheat germ agglutinin. *K. Harada, Japan.*

*Glycopinion articles:*

Evaluation of recombinant glycoproteins. *K Seamon, USA.*

Inositol acylation of glycosylphosphatidylinositol membrane anchors: what it is, and why it may be important. *M C Field, USA.*

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## Journal of Muscle Research and Cell Motility

***Editors: C C Ashley, University of Oxford, UK, C R Bagshaw, University of Leicester, UK. J. Sellers, National Institutes of Health, USA, P Sheterline, Liverpool University, UK, A P Somlyo, University of Virginia, USA, R T Tregear, AFRC Institute of Animal Physiology and Genetics Research, UK***

The **Journal of Muscle Research and Cell Motility** publishes original research papers on all aspects of muscle, contractile systems and cell motility. The Journal favours papers with a molecular bias in the field of cell motility and papers on non-muscle contractile systems and microtubule-based motility. It maintains a rapid publication schedule, and exceptional papers are expedited.

**The Journal is covered by *Current Contents, Index Medicus* and *Excerpta Medica*.**

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## s in Fish Biology and Fisheries

***ny Pitcher, Renewable Resources Assessment Group, Imperial College, on, UK***

**iews in Fish Biology and Fisheries** is a quarterly international journal devoted to publishing portant review articles on any aspect of fish and fisheries biology. Reviews are accepted in any field of h biology where the emphasis is on the whole organism. The subjects covered include physiology, olutionary biology, taxonomy, zoogeography, behaviour, ecology and exploitation. The reviews provide up-to-date synopsis for research workers in the chosen field with an outline of the next problems hich should be tackled. They also provide the non-specialist fish biologist with an awareness and derstanding of the field.

addition to review articles, details of interesting papers given at recent conferences, book reviews and otices of forthcoming conferences are included.

he Journal caters for all those with an interest in fish biology and fisheries including those from iversities and research institutes, fishing industries, local, regional and government institutions and ternational organizations.

*SN: 0960-3166 Volume 3 (1993) 4 issues*
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## NEW IN 1993

## Aquaculture International

The Official Journal of the European Aquaculture Society

***Editor: M Poxton, Heriot Watt University, UK***

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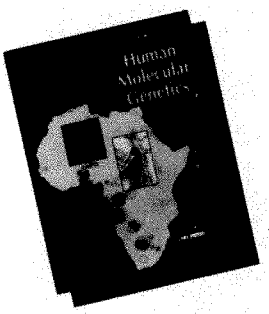
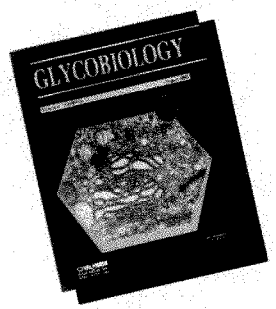



## ☐ Human Molecular Genetics

Edited from Oxford and Stanford by Kay Davies and Huntington F Willard, this major new journal publishes the best original research on all aspects of human molecular genetics, within a maximum of three months from receipt of manuscript.

Areas of interest include the analysis of the structure and function of human genes, the molecular basis of human inherited disease and disease predisposition, chromosome structure and function, developmental genetics, molecular aspects of cancer genetics and genetic therapies. The molecular analysis of other species is also of interest where such studies are relevant to the understanding of the human genome.

*Vol. 1, monthly from April 1992, ISSN 0964-6906*

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Recent international acceptance and usage of the relatively new term 'glycobiology' reflects the rapid and continuous growth of research into one of the last great frontiers in biochemistry. **Glycobiology** provides a unique forum dedicated to research into the structure and function of glyconjugates.

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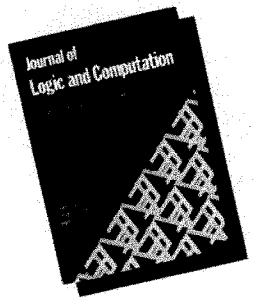


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# Drugs and the brain

S. J. Enna

**Molecular Neuropharmacology.** Editor-in-chief G. N. Woodruff. *Macmillan. 4/yr. £115.*

As an integrated discipline, pharmacology has been quick to incorporate the tools of the biochemist and molecular biologist in its quest to define the mechanism of action of therapeutic agents. Neuropharmacologists have been particularly eager to use these approaches because the brain is less amenable to traditional study than other organ systems. With the growing interest in neuroscience, the number of papers dealing with the molecular basis of drug action has increased dramatically. *Molecular Neuropharmacology* was launched in mid-1990 as a vehicle for disseminating such information. Manuscripts covering this speciality have historically been scattered among several primary journals, including *Molecular Pharmacology, Journal of Pharmacology and Experimental Therapeutics, European Journal of Pharmacology, Brain Research* and *Journal of Neuroscience*. Although each is recognized as having high editorial standards, none is specifically for neuropharmacologists. The chief journal in this regard is *Neuropharmacology*, which has a broader mandate than *Molecular Neuropharmacology*. So it would seem that this offering addresses a real need and, given the number of excellent workers in the field, has the potential to develop into a primary journal. This possibility is enhanced by the fact that the editorial board is composed of an international group of highly acclaimed neuroscientists and neuropharmacologists.

During the first two years of publication, four issues of *Molecular Neuropharmacology* have appeared. Eleven months elapsed between the first and second, with three months separating the third and fourth issues. The quality of the articles is generally good, many contributed by leaders in the field. But as is the case for most new journals, it would seem that these authors are still submitting their best work elsewhere. Nonetheless, if *Molecular Neuropharmacology* can begin publishing on a regular schedule and continue to attract authors of this calibre, it should ultimately find a loyal audience.

Given its stage of development, price and past publication record, it is difficult at this time to recommend the journal for individual subscribers. It certainly has a place in biomedical libraries, as the quality of the published articles is such that they will be cited in the primary literature — access to the journal will therefore be required by many investigators. □

*S. J. Enna is in the Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66160, USA.*

# Going like a dream?

Eric A. Barnard

**Current Opinion in Neurobiology.** General editors Albert Aguayo and Martin Raff. *Current Biology. 6/yr. £209.95, $445 (institutional); £69.95, $129.95 (personal); £29.95, $49.95 (student).*

A FAMOUS newspaper once claimed to contain "all the news fit to print" — a claim long since outdated as far as the 'all' is concerned, not to mention the 'fit'. Similarly, the claim of this journal that it reviews "all advances" in "the entire field of neurobiology" and gives a "comprehensive listing" of the literature can be taken as an impossible dream. The journal divides the field into main sections, one per issue, repeated cyclically every year: development; cognitive neuroscience; signalling mechanisms; sensory systems; neuronal and glial cell biology (half an issue) and disease, transplantation and regeneration (half an issue); and neural control. The issues are necessarily selective, each consisting of a set of 'topics' or brief reviews, usually four pages in length, which inevitably cannot include all the advances of the past year.

For example, in the issue on signalling mechanisms we find the topic of G-protein-coupled receptors. That is hardly a surprise, but this year's review of that topic is confined to dopamine receptors (albeit done well and comprehensively, by D. K. Grandy and O. Civelli), and the bibliography there of "the papers of the greatest interest over the previous year" is also confined to these receptors. Therefore, less than one per cent of the G-protein-coupled receptors can actually be reviewed in one year of this journal.

Let it be said immediately, though, that the scientific standard of the journal in general is very high, and the overall editing of each issue is done with care and insight. The ability of the section editors to get many experts to write the almost uniformly excellent reviews, and to do so in a timely manner, is creating a success for this considerable enterprise.

Review journals for the neurosciences are certainly becoming more numerous. Do we need another one? Of the conventional type, most of us would think not. The same is true of the *Trends* type. *Current Opinion*, however, is something else again. It uniquely fulfils a need. First, by means of the planned annual cycle of reviews, readers know in which issue, for example, glial cells or neurodegenerative disease or cognitive systems will be covered. They need no longer scan the contents lists of a variety of review journals to see what the lottery of topic selection has brought up.

Second, there is an exceptionally wide coverage of fields, although it does seem to me that the balance struck underrepresents the proportion of publications on molecular aspects. This is exemplified by the lack of space for reviews of most G-protein systems and of the molecular biology of the glutamate receptors (only the sections recur each year, not all the designated topics within each). Examples of topics unrepresented this year in the signalling issue are potassium channels, transmitter transport, ionic exchangers and pumps.

Third, each issue contains a "complete bibliography of world literature" on the fields the issue embraces. The list covers one year, ending five months before the publication date, but is not quite as comprehensive as claimed. Sampling just some of the topics in the 82 pages of bibliography for the excellent June 1992 issue edited by P. Ascher and C. Stevens on signalling mechanisms, I found several relevant articles missing. Established journals not covered include *European Journal of Pharmacology, Molecular Pharmacology, The New Biologist, Biochemical and Biophysical Research Communications* and *FASEB Journal*; instead, some less-read journals are used. The field of neurotransmission is only partially covered.

These limitations can and should be partially remedied. In addition, a distracting practice is the requirement for each reviewer to rate the papers cited for their interest; two points, one point or (for most) none. This is appropriate for a restaurant guide, but I feel it is invidious here. One or two reviewers bestow a good proportion of the few two-star ratings on their own papers. Some avoid one-star ratings; others completely omit two-stars, presumably owing to an understandable embarrassment. I urge the editors to drop this highly subjective system. Reviewers wishing to draw attention to a paper can do so with more clarity in the text of their articles. And it would be helpful to non-experts

if all the relevant review articles published elsewhere on each topic were listed together.

These points would improve a series that is likely to become an indispensable resource for many libraries, as well as for individuals who are particularly interested in one or more of the main section areas. □

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# Better read than dread

*Kenneth S. Kosik*

**Brain Pathology.** Editor-in-chief Paul Kleihues. *International Society of Neuropathology, PO Box, CH-8033, Zürich, Switzerland. 4/yr. SFr120, $90 (institutional); SFr75, $50 (personal).*

THIS journal has a stylish, albeit somewhat lugubrious cover on which the image of a microscopic section, often from a dread brain condition, is surrounded by a black border. Inside, each issue is devoted to a single chosen theme for which a guest editor has solicited reviews (unsolicited papers on the chosen topic are also considered). In the evolution of scientific journals, the appearance of the review journal is a relatively late development. Its arrival represents a confidence that the flow of information in a particular field is sufficiently large. The editors of *Brain Pathology* and its publisher believe and, indeed, have so far demonstrated, that neuropathology has achieved this evolutionary milestone. *Brain Pathology* has tackled the somewhat staid world of neuropathology, with its usual emphasis on descriptive morphology, by inviting contributions on the many intriguing molecular and cellular aspects of brain pathology. Whereas the literature on the neurosciences is voluminous and burgeoning, neuropathology as a distinct field has not grown so quickly. This journal, as the first theme-oriented journal devoted to neuropathology, hopefully heralds a change in this relatively slow growth.

The themes or symposia (as they are called in the journal) address topical subjects for which there are now a great deal of data. Some of the recent themes are: "Brain tumours: Molecular aspects", "Mitochondrial encephalomyopathies", "Gene transfer into the nervous system", "HIV infection of the brain" and "Alzheimer's disease: Molecular basis of structural lesions". The contributions are all extremely relevant and the contributors are scientists of high repute. The organization of each symposium is left to the discretion of the guest editor, and in each case the reviews have succeeded in being up to date, although not always comprehensive.

Historical notes spice many of the papers, such as that on "Neuropathology in the Third Reich" (neuropathology is more conscious of its past than many other scientific disciplines). The journal is rounded out with meeting reports, meeting abstracts, book reviews and portraits of now legendary figures such as Lucien Rubinstein and Harry Zimmerman. Reflecting the views of the International Society of Neuropathology, *Brain Pathology* is admirably international, at least to the extent that North America, Western Europe and Japan are well represented. The editors have the well-intentioned wish to extend the circulation of the journal to South America and Africa, which at present account for only seven and two subscriptions respectively. One step in this direction is the low cost of a subscription made possible by the desktop publishing techniques used by the journal. Nonetheless, the format is attractive and the photography (including colour) is of very high quality, as is mandatory for a neuropathology journal. The recent decision by the editors to accept primary research papers will certainly determine whether the journal succeeds in the long term. □

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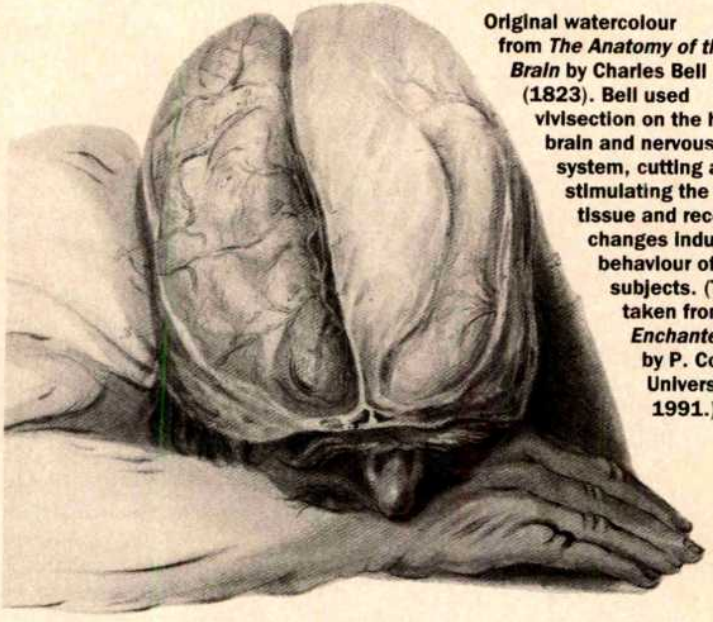

**Original watercolour from *The Anatomy of the Brain* by Charles Bell (1823). Bell used vivisection on the human brain and nervous system, cutting and stimulating the living tissue and recording the changes induced in the behaviour of his subjects. (The picture is taken from *The Enchanted Loom* edited by P. Corsi, Oxford University Press, 1991.)**

# Established newcomer

*Larry W. Swanson*

**Frontiers in Neuroendocrinology.** Editors W. E. Ganong and L. Martini. *Raven. 4/yr. US and Canada $215, elsewhere $260 (institutional); US and Canada $162, elsewhere $190 (personal).*

A YEAR ago I was asked to review a promising new addition to the literature, *Journal of Neuroendocrinology*, and I am now doing the same for a well-established 'new' journal, *Frontiers in Neuroendocrinology*. I say well-established because it actually began in 1969 as a book of review articles published biennially. At the time, the editors (who remain valiantly in place) observed that neuroendocrinology is concerned not only with neural control of endocrine secretion but is a truly broad yet fascinating discipline concerned with mechanisms critical for survival of the individual and the species.

This series, at the interface between endocrinology and neuroscience, was so successful that in 1990 it began quarterly publication of three or four reviews, most of which are commissioned. From the beginning, *Frontiers* built a well-deserved reputation for publishing interesting, current, authoritative reviews in the range of 15 to 50 pages, and many readers looked forward to its appearance. So it seems almost inevitable that a more timely publication schedule would eventually be adopted, and the current

format is an innovative, useful solution. In addition, the quality and breadth of the journal seem assured by the help of a truly first-rate editorial board, and the personal subscription rate is reasonable enough for many workers to be able to have their own copy. Perhaps in time, *Frontiers in Neuroendocrinology* may assume a stature comparable to that of the venerable *Physiological Reviews*, though on a more limited scale. □

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# Blood relation

*Stan Heptinstall*

**Blood Coagulation and Fibrinolysis: An International Journal in Haemostasis and Thrombosis.** Editors J. L. Francis and S. G Gordon. *Rapid Communications of Oxford. 6/yr. £240, $456 (institutional).*

THE cover of this journal — glossy black background overlaid by yellow lines (symbolic of fibrin, the end product of the coagulation cascade), red dots (red cells) and white letters (depicting some of the factors involved in coagulation and fibrinolysis) — is, at first sight, striking. But like a necktie (of similar colours) that I bought to make an impact, it soon loses its initial effect. The initial impression of the journal's contents, though, should prove to be much longer lasting.

The stated aim of the journal is to provide "a fast new medium in haemostasis and thrombosis". For the non-specialist this can be interpreted as 'fast publication in a new journal of information on clinical, laboratory and experimental aspects of bleeding and clotting'. 'Fast' was to be within 30–60 days of a paper's acceptance. The information was to be in the form of original research articles, state-of-the-art reviews, short reports, technical notes, case reports, reports of meetings and letters to the editors. Book and computer-software reviews and a meetings diary section were also to be included. Proceedings of meetings were to be reproduced either in the journal itself or in special supplements. To what extent has the journal succeeded in these aims?

Publication within 30–60 days from acceptance has not yet been achieved, but in 1991 (volume 2) the average time was only 72 days and it seems that the times are getting shorter. Perhaps more importantly, in 1991 the average time for a decision to publish was only 33 days — surely time to publication does not really matter to most of us as much as publishers think. Is it not more important to get feedback from the editors and referees so that their comments and criticisms can be considered and papers amended accordingly?

The editors have succeeded in publishing the full range of contributions planned for and the quality has mainly been good. The number of book and software reviews has been phenomenal. The journal has appeared regularly at bimonthly intervals and one additional supplement has been produced. Although there are other journals concerned with haemostasis and thrombosis, this new one does provide an alternative home for what is an expanding area of research.

The editors are to be congratulated on their efforts so far. But I do urge the publisher to consider a change in cover design, to use lighter paper and to reduce the size of some of the figures and tables. This would bring a sigh of relief from those of us who take our reading home. □

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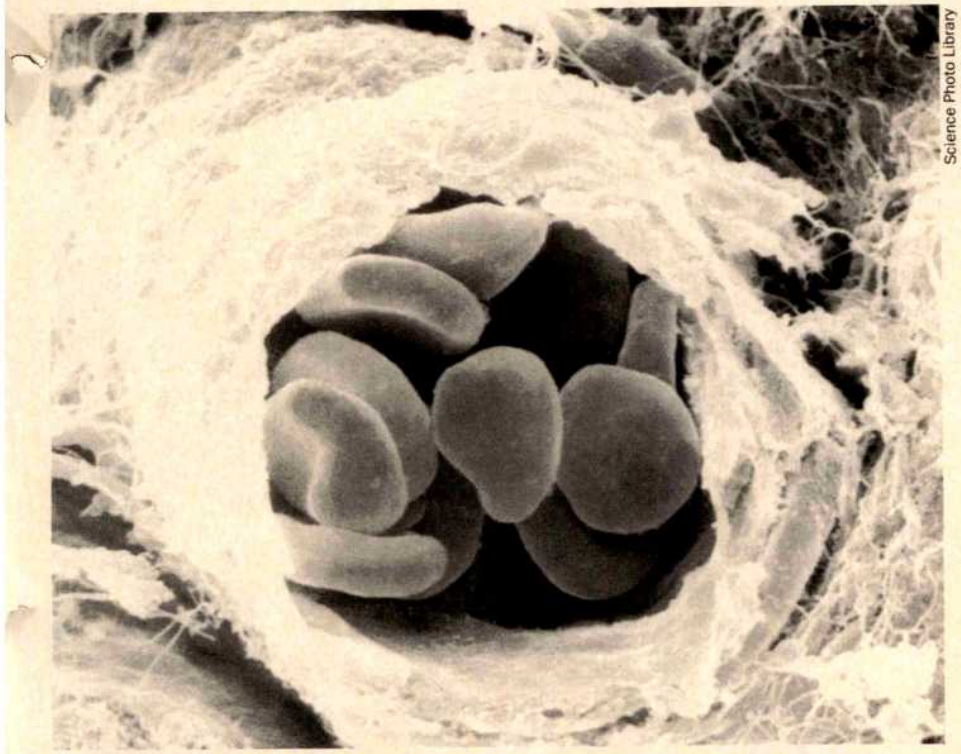

Science Photo Library

**Going by tube — scanning electron micrograph of erythrocytes in an arteriole (× 3,245).**

# Beauty and the bible

*Gobinda Sarkar*

**PCR: Methods and Applications.** Editor Judy Cuddihy. Associate editors D. Bentley, R. Gibbs, E. Green and R. Myers. *Cold Spring Harbor Laboratory Press. 4/yr. US and Canada $170, elsewhere $180 (institutional); US and Canada $55, elsewhere $65 (personal).*

"THE philosophy of a scientist is to make the world a simpler place." I once tried to impress this upon my mother after returning from a late-night liaison with a log-phase bacterial culture. She responded with a queer look of unconcealed scepticism. Then I told her about Kary Mullis and his polymerase chain reaction (PCR). I explained that PCR is so simple and powerful that it can perform many tasks faster and more efficiently than bacteria and that it has made possible things that were unthinkable even a few years ago. She held my hand and almost implored, "I know you are not Kary Mullis, but you sure should use his 'chain reactor'. That way you'd not have to spend late hours in the lab." Although my mother was born at a time when female education was discouraged, she could understand the very essence of PCR. PCR is that simple.

PCR is so simple that it is beautiful. No wonder everybody turned into admirers in no time. But there is always a risk of a beauty being maligned by over-adulation. Fortunately, before this could happen, *PCR: Methods and Applications* came up with all the care and protection that this awesome beauty

deserves. The journal contains reviews, research papers and technical tips on PCR-based methods and comes from a publishing house of unquestionable credentials. Readers will therefore have certain expectations, which, I believe, have been met with a robotic consistency of high-quality papers. The list of associate editors and the editorial board contains names that would be the envy of many publications. With assistance from such stars, it is relatively easy for Judy Cuddihy, the editor, to bring "focus and quality control" to the wide-range of PCR developments. Indeed, the relatively low publication frequency of the journal may also be helping to achieve this quality control. It seems that if PCR is considered a religion, *PCR: Methods and Applications* is its bible.

But we have come to expect more. Although the journal does a good job by providing a short list of selected articles on the cover, it is difficult to form a link between the cover and the contents because all the issues look alike. And it might be a good idea to include a list of *all* the previous articles in every issue (or a modification of this theme). This might transfer the journal from the office to the workbench, which is where a methods journal is supposed to be. Finally, the journal would benefit from a correspondence section to provide a vehicle through which sellers and consumers can communicate. The personal subscription rate is reasonable, but could have been a little less considering the worldwide hunger for PCR. Perhaps there should also be a reduced rate for developing countries as well as for — unprecedented though it may be — the new European countries.

I have a feeling that there is a limit to the number of new PCR-based methods that can be developed, although new applications are going to continue to arise for a long time. Assured, in the meantime, that our beauty is in able hands, we can now go back to the bench and devour the bible. □

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**Journal prices**

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1993. This information may not be complete in all cases. Readers interested in a particular journal are therefore advised to check prices with the publisher before subscribing.

# Breasting the flood

*David Blow*

**Current Opinion in Structural Biology.** General editors Wayne Hendrickson and Aaron Klug. *Current Biology. 6/yr. £264, $445 (institutional); £69.95, $129.95 (personal); £29.95, $49.95 (student).*

THE continued acceleration of scientific discovery, most of all in the biological sciences, brings problems for us all. We can cover the original literature only within an ever-narrowing field. The *Current Opinion* series of journals, of which there are now six in biological sciences and sixteen in clinical medicine, provide a carefully designed scheme for current awareness within broader specialities, which have now become far too large for one to read, or even scan, all the publications.

For structural biology, for example, coverage is divided under 13 separate sections, of which about half are descriptive (proteins, nucleic acids) and half analytical (catalysis and regulation, protein engineering and design). The two general editors choose one or two people to edit each of these sections. The section editors invite eight or ten review articles or half-a-dozen pages on specific topics, and add a brief editorial overview. Each section is reviewed annually.

To ensure coverage of the current literature, *Current Opinion in Structural Biology* organizes the scanning of some 60 journals, and each issue lists all the publications in the past year that are considered relevant to the sections under review, whether or not these are cited in the reviews.

The success of such a scheme depends partly on a robust organization, but above all on the quality of the individual reviews and their authors. The editors of *Current Opinion in Structural Biology* have recruited scientists of outstanding international reputation as section editors, who in turn have chosen excellent reviewers. There must be difficulties of coverage, in preventing both gaps and overlaps from becoming too serious, but these are not obvious.

Most articles are interesting and informative, with a definite theme and often a point of view to present. They are not limited to citing the current literature and do not slavishly cite every paper in the field, nor do they avoid citing the same paper in different articles.

My praise should not be fulsome. These reviews are written by busy people with deadlines to meet. If you have specialist knowledge, you will find the usual irritating imperfections of reviews: misinterpretation, omission, ignorance of precedence, acceptance of unfounded claims, hitching to the bandwagon. But the publication is a stimulating and, above all, a useful one. □

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# Structure and function

*J. A. Littlechild*

**Protein Science: A Publication of the Protein Society.** Editor-in-chief Hans Neurath. *Cambridge University Press. 12/yr. US and Canada $550, elsewhere £310 (institutional); US and Canada $130 (personal).*

ANOTHER journal appearing on protein structure and function will produce some dismay among many researchers. Why do we need yet another journal to add to the ever increasing list that we expect our libraries to subscribe to with their ever decreasing funding? There are already other relatively new journals, such as *Proteins: Structure, Function and Genetics*, that cover the general field of protein research.

A multidisciplinary approach is necessary to understand the diverse aspects of protein structure and function. *Protein Science* clearly addresses this need. It seeks to report all aspects of protein research, covering structural determination and other biophysical, chemical and recombinant methods. The overall presentation is excellent and the coloured figures are of good quality. Because the journal is funded in part by the American Society for Biochemistry and Molecular Biology and by the Innovative Technology Fund supported by the Biophysical Society, the subscription rates are good value, there are no page charges and there are reduced rates for colour prints (so necessary to show and display protein structure). Also, the journal has adopted the new approach of presenting extra data and pictures of protein molecules on a computer disk included with each issue. Hopefully, libraries will have the facilities to make full use of this information, which also now includes tutorials that should prove invaluable for teaching purposes. This new approach is funded by the Innovative Technology Fund, and is expected to develop as the journal becomes established.

Although associated with the American Protein Society, the journal seeks to be international and has a fair representation of articles from both European, non-European and US researchers. The personal touch imparted by a monthly "Recollections" article provides reminiscent reading for more established workers and stimulating reading for younger scientists. In addition, a special section entitled "For the Record" features one-page refereed reports on recent developments. The journal's future looks bright in view of its association with the Protein Society, although I do feel that its news and views on the society caters more for the American than the non-American research worker. But overall the journal will be of use to all researchers interested in protein science and is well deserving of a lasting place among the new journals covering this growing area of research. □

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# Reading between the vines

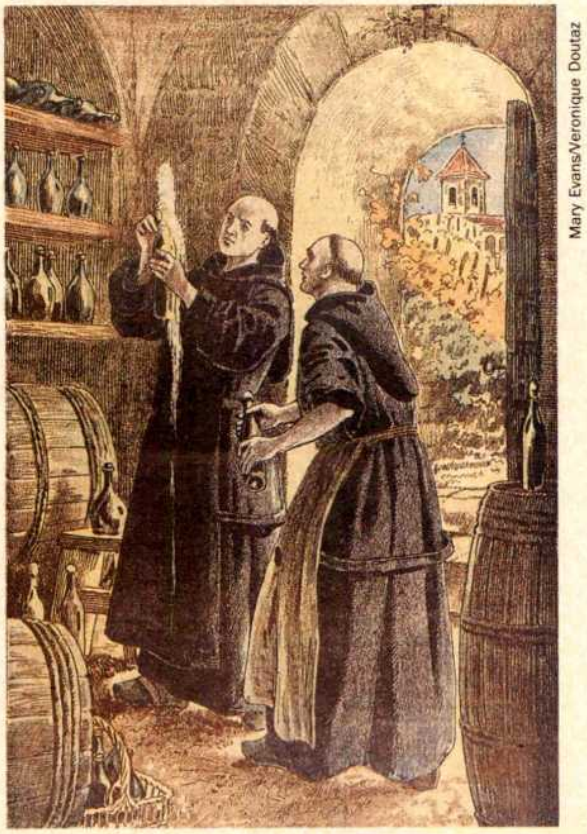
*Robin Goswell*

**Journal of Wine Research.** Editors Jasper Morris and Tim Unwin. *Carfax. 3/yr. US and Canada $298, Europe £148, elsewhere £177 (institutional); US and Canada $107, Europe £48, elsewhere £61 (personal).*

THIS journal was founded by the Institute of Masters of Wine, an association of professionals working in the UK wine trade who are dedicated to maintaining a high level of expertise, primarily by organizing examinations and conferences. The journal is international and multidisciplinary, publishing the results of recent research on all aspects of viticulture, oenology and the wine trade. It aims to "enhance and encourage scholarly and scientific interdisciplinary research". The editorial and international editorial boards include many distinguished academics and members of the trade. In the two volumes, each of three issues, the editors succeed in obtaining high-quality contributions on a range of topics including practical wine making, viticulture, wine medicine, chemistry, microbiology, geography, geology and history. Papers come from as far afield as Argentina, Australia, Bulgaria, France, Greece, New Zealand, the United Kingdom and the United States.

Of course, most wine-producing countries have their own scientific or technical journals relating specifically to the wine industry. These tend to cover wine chemistry, microbiology, viticulture, practical wine making and economics, but with few exceptions they devote more than 90 per cent of their contents to the national industry and the work of locally based researchers.

A multidisciplinary journal will succeed in its aims only if it earns respect for the quality of the contributions, and if most of its readers and contributors are able to read the majority of the articles with sufficient understanding to gain some stimulus for their own ideas. The quality of the contributions does not seem to be in doubt. The microbiological and chemical articles, the subjects with which I am most familiar, seem to be similar to those appearing in other highly reputable journals. But the highly technical vocabulary used may cause difficulty for those trained in other disciplines. For this reason, the editors might be well advised to seek review-type articles as well as reports of original research. Such a policy would certainly not downgrade the scholarly standing of the journal.



Mary Evans/Veronique Doutaz

**Uncorking a winner — Dom Pérignon (1638–1715), a Benedictine monk, discovers the secret of champagne.**

Publication of articles seems to be commendably quick, and the presentation of the journal is good. The cost for both individuals and institutions is very much what one would expect under current economic conditions. Altogether, *Journal of Wine Research* is a worthy enterprise that deserves to succeed. □

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# Technical triumph

*T. A. Connors*

**Toxicology Methods.** Editor-in-chief Shayne C. Gad. *Raven. 4/yr. US and Canada $137, elsewhere $158 (institutional); US and Canada $84, elsewhere $97 (personal).*

REVIEWERS of new journals in *Nature* are offered guidance on what should and should not be included in their review. We should not moan unduly about the ever increasing number of journals, for example; and we are told that the most important question to be asked about a journal is whether it fills a gap, providing a focus for an emerging discipline or cross-discipline. I believe *Toxicology Methods* does fill such a gap, although some people will argue that toxicology has already emerged as a discipline and, to prove it, use words like 'toxicokinetics' instead of the more comfortable 'pharmacokinetics'.

But what is beyond doubt is that toxicology is a cross-disciplinary subject that will continue to grow for the foreseeable future. The introduction of more and more regulations relating to chemicals potentially hazardous to humans, animals, plants and the environment stimulates applied research and the development of tests for risk assessment. These tests often uncover new biological properties of chemicals, which in turn lead to basic studies on mechanisms.

The editor acknowledges that many of the techniques in toxicology have arisen from recent developments in biochemistry and cell and molecular biology, but there is no doubt that increasingly there are techniques and methods invented by toxicologists for the specific needs of toxicologists. Although toxicology jour-

nals that publish original research have been around for a long time, this is the first to devote itself to new methods, and that is why it fills a gap.

The first volume covers a range of subjects and includes three excellent technological reviews on quantitation of cell proliferation, whole-body autoradiography and *in situ* hybridization. The original papers are roughly divided between *in vivo* and *in vitro* methods and new analytical techniques. Not all of the methods described are brand new, but they do represent important advances. A. Barlow *et al.*, for example, have taken a second look at an *in vitro* test that assesses the corrosive potential of chemicals. This is carried out by measuring changes in transcutaneous electrical resistance, but the method is flawed because organic solvents and surfactants that are not corrosive cause similar changes. Simply swapping the standard sodium chloride electrolyte with magnesium sulphate reduces the false positives from 26 to 9 per cent. Not great science perhaps, but a considerable improvement on an important test. In its first year, the journal has had a 27 per cent rejection rate and the time from receipt of a paper to its publication has averaged three-and-a-half months.

For a new journal, the quality of the original papers is good and is bound to improve as the journal becomes better known by toxicologists. □

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# Burgeoning biocontrol

*Keith Sunderland*

**Biocontrol Science and Technology.** Editor-in-chief Chris C. Payne. *Carfax. 4/yr. US and Canada $280, Europe £140, elsewhere £160 (institutional); US and Canada $99, Europe £46, elsewhere £57 (personal).*

**Biological Control.** Editors-in-chief Raghaven Charudattan, W. Joe Lewis, Harry K. Kaya and Charlie E. Rogers. *Academic. 4/yr. US and Canada $146, elsewhere $166.*

THESE two new journals, launched in 1991, seem at first sight to be in competition for the same market. I looked for signs of 'niche differentiation' in their first volumes, but found little evidence of any. Both have responded to the need to collect together biocontrol papers previously scattered in a wide range of journals, and both can justifiably claim to be multidisciplinary journals with high scientific and editorial standards and a good quality of production. There are some minor differences in the facilities offered: *Biocontrol Science and Technology (BST)* has a contents section and author index for each volume, whereas *Biocontrol* has, in addition, a subject index and list of referees and will publish symposium proceedings and letters to the editor. On the other hand, *BST* has a regular review slot and accepts short communications for rapid publication.

Perhaps the main difference lies in geographical coverage; *BST* has lived up to its promise to be truly international (no more than 5 out of the 27 members of the editorial board come from a single country and no more than 28 per cent of the 28 papers in the first volume emanate from one country). *Biocontrol* makes no claim to be international; 21 out of 33 members of the editorial board live in the United States and 87 per cent of the 47 papers published in the first volume are also from that country.

Both journals have an extensive remit, covering the theory and application of biological control of animal pests (including pests of plants and arthropod pests of man, our stored products and domestic animals) and of diseases and weeds; this is a strength, admitting the possibility of cross-fertilization of ideas between subdisciplines. A good balance of papers has been achieved, whether judged in terms of type of biocontrol agent (including microbes, nematodes, arthropods and molluscs), target pest or category of study (including the biology, ecology, physiology, genetic improvement, culturing, quality control, application and effectiveness of control agents). Currently, *Biocontrol* has slightly more emphasis than *BST* on the ecological aspects of biocontrol.

The field of biocontrol is growing rapidly, driven mainly by the global trend towards reduction in the use of chemical pesticides and the need to develop substainable and environmentally acceptable alternative strategies of pest control. And the accelerating spread of pest species beyond their countries of origin demands greater allocation of resources to the study of biological control. Given this situation, it seems likely that there will continue to be more than enough material to sustain both of these journals. □

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# Seeds of success

*Robin Probert*

**Seed Science Research.** Editor M. Black. *CAB International. 4/yr. US $214, elsewhere £116.*

SEEDS provide 55 per cent of the daily per capita energy requirement of humankind, excluding their contribution to the nutrition of livestock. The importance of seeds is reflected in the amount of research devoted to them. For example, a recent literature search revealed more than 2,400 reports in the past 21 years alone in which seed germination and dormancy were linked to temperature — a subject reflecting only a small part of the spectrum of seed-related issues.

Because of the convenience of seeds, researchers often turn to them for a model system for investigating central issues in plant science. In his opening address to the Fourth International Workshop on Seeds (July 1992, Angers), C. M. Karssen described this activity as "science with seeds", as distinct from "seed science", which may be defined as


**From top to bottom: seed pod of the field penny cress, *Thlaspi arvense*; germination of clover seeds, *Trifolium* sp.; and seeds of the Moreton Bay chestnut, *Castanospernum australe*, from the rainforests of Australia.**

an integration of different disciplines in an attempt to explain seed behaviour. The different approaches to seed science alone range from plant ecology to molecular genetics, and in the absence of a quality journal devoted to fundamental studies on seeds, publications have tended to be scattered throughout the plant science literature.

There are a few journals devoted to seeds, but these concentrate on applied aspects. So, to provide a single forum for the publication of high-quality basic research, *Seed Science Research* was launched in March 1991. In its first five issues, there have been two very different but equally good reviews; 36 research papers of mostly high quality, covering a range of topics from seed development to ageing; one very welcome exchange of conflicting opinion between two prominent research groups; and seven book reviews. Publication times have been impressive, with most papers appearing within six months of submission and some within three to four months. Of course, this may reflect a paucity of manuscripts — several important papers have appeared in other journals since the launch of *Seed Science Research*, suggesting that a number of researchers are 'sitting on the fence', waiting perhaps for the reputation of the new journal to become firmly established.

Recent approaches to the study of seeds, such as the use of hormone-deficient mutants and the analysis of gene expression, herald advances that could have profound implications for future crop development and biodiversity conservation. *Seed Science Research* is a logical vehicle for such basic research and the maintenance of its present high standards, through the continued (and hopefully increased) support of mainstream seed biologists, should secure its position as the principal journal for the advancement of seed science. □

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# Casting the net widely

*J. H. S. Blaxter*

**Reviews in Fish Biology and Fisheries.** Editor Tony J. Pitcher. *Chapman and Hall. 4/yr. US and Canada $185, Europe £99, elsewhere £110 (institutional); US and Canada $59, Europe £32, elsewhere £32 (personal).*

THIS journal, edited by T. J. Pitcher of Imperial College London with a technical editor and an editorial board of 19 well-known scientists, is dedicated to publishing review articles on any aspect of fish and fisheries biology, with an emphasis on adaptation, function or exploitation. The "Aims and Scope" declared on the inside front cover, however, list a series of topics that cover almost every conceivable subject: evolutionary biology, zoogeography, taxonomy, including biochemical taxonomy and stock identification, genetics and genetic manipulation, physiology, functional morphology, behaviour, ecology, fisheries assessment, development (ontogeny), exploitation, aquaculture and conservation. In fact, almost everything is apparently included except cell biology, biochemistry, disease and parasites, and pollution.

In his leading article in the first issue, Pitcher evades defining the word 'fish', leaving the series open to "animals which live in water and are harvested by man". These rather strange open-ended limits to acceptable topics seem to have been breached in the first three issues, where some of the reviews are strongly biased towards species that are certainly of little commercial value. Indeed, they are probably exploited only by research workers.

So far there have been three or four reviews of 20–30 pages in each issue. They are brisk and tend to deal only with the more recent literature. The subject matter covered is wide, with some signs of lack of an editorial master-plan. For example, there are two reviews on the role of catecholamines in controlling breathing of fishes, written by two different groups of authors and apparently supporting different theories. There is astonishingly little over-lap in their two reference lists. It would have been much more helpful if these two reviews had been brought together.

Other reviews cover fish disease as a modulator for marine pollution, alarm signals, solitary chemosensory cells, fish swimming, marine reserves for managing reef fisheries, amphibious fish and death rates; there is an especially innovative review of cannibalism in fish, with a very useful bibliography.

The editor has spread his net wide, perhaps a little too wide, in these early issues. A little more coherence within each issue might have been a good idea. The editor's aim of "an up-to-date synopsis of work and insight of the major issues currently occupying research workers" seems to have been achieved. Whether it is possible to both "give specialists a brief but comprehensive review of developments" *and* "provide the non-specialist with awareness and understanding" remains to be seem.

In summary, this seems to be a promising journal, with room for some evolution of content, style and length. The editor is to be congratulated if he can maintain an output of four issues a year in the coming years. □

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**Watercolour of a gurnard by J. W. Turner (1775–1851). The picture is reproduced from *A Book of Sea Creatures*, published by the Victoria and Albert Museum, London, which features some of the most striking creatures, both real and fantastic, from the museum's collection. Price £4.95.**

# Slippery slope

*Ellis L. Yochelson*

**Ichnos: An International Journal for Plant and Animal Traces.** Editors S. George Pemberton and Robert W. Frey (dec.). *Harwood Academic. 4/vol. $300 (institutional), $107 (personal).*

DURING this generation, the study of traces of past organic activity has been developed to a fine art by some palaeontologists, and this new journal puts their best foot (or footprint) forward. About two-thirds of the printed pages are "Research Articles". "Short Communications" include brief papers, book reviews, discussions of techniques, memorials and a few other items. All the material is well edited and adequately illustrated. The age of specimens discussed ranges from Precambrian to present-day. It is interesting stuff for those who like burrows, borings, trails and other indications of the 'work of animals' and of plants. The science reported is just fine. Now having praised, I shall damn.

In physical appearance, the journal invites comparison to the *Journal of Paleontology*. Both are printed with two columns to a page in the large format that is becoming standard for many journals. Both are printed on coated stock and the photographic illustrations and line drawings of both are reproduced well. *Ichnos* has 54 lines to the column, *Journal of Paleontology* has 66, plus a few more characters per line. Centred headings are more prominent in *Ichnos*, with more white space above and below. In *Ichnos* all articles begin on a right-hand page. As a result, of the 330 pages in four issues of *Ichnos*, 28 are blank. In considering whether to purchase a journal, some people work out the price per page, and even per character. This empty space all adds up to the detriment of the journal.

It is understandable that each new discipline or subdiscipline in science wants to establish its own independence. But there are at least four long-established journals in palaeontology to which virtually all the material in *Ichnos* could have been submitted; none of them has an exceptionally large backlog. A specialist journal weakens the more general journals in the field. It may be counterproductive in having the effect of isolating new subdisciplines from a wider audience. There are only about 5,000 palaeontologists and there is a limit to the number of journals that these individuals and libraries can support.

Profit is not a bad thing, but gross profit is. A few years ago, the American Association of Research Libraries issued a strong condemnation of commercial journals for price-gouging. To keep down costs, they urged scientists to publish their own journals. Had the ichnyologists followed this path, they would be receiving more support in this review.

**Dinosaur tracks at Glen Rose, Texas. (The picture is reproduced from *Global Warming* by A. Revkin, American Museum of Natural History Environmental Defense Fund, £20.)**

Co-editors S. George Pemberton and the late Robert Frey worked hard for many years to establish a journal and they have produced a good-quality product. Unfortunately, the richest science libraries today will not take a new journal unless an old one is discontinued, and most are dropping journals. In this economic climate I cannot recommend that *Ichnos*, or any new commercial journal, regardless of its quality, be purchased by a library. □

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# NEW & NEWLY ACQUIRED JOURNALS

## Engineering, Materials Science, Energy & Automation

### APPLIED SUPERCONDUCTIVITY

Editor-in-Chief: **Roger B. Poeppel**, *USA*

This new monthly journal, a section of *Solid-State Electronics*, will deal with both large and small scale applications of superconductivity, including, particle accelerators, magnetic levitation systems, electromagnetic launchers and storage devices among others. Articles describing unique processing technologies and performance characteristics will also be published. *Applied Superconductivity* and *Solid-State Electronics* are both available separately and are also available in a joint subscription which will save you more than 10%.

1993: Volume 1 (12 issues) ISSN: 0964-1807 (00101)
Annual Subscription 1993 £345.00 US$656.00*

### CONTROL ENGINEERING PRACTICE

A Journal of IFAC, the International Federation of Automatic Control

Editor-in-Chief: **M. G. Rodd**, *UK*

*Control Engineering Practice (CEP)* publishes papers which illustrate the direct application of control theory and its supporting tools in all possible areas of automation. In addition to purely technical applications papers, *CEP* carries material covering topics linked to the application of automation, including social effects, cultural aspects, project planning and system design, and economic and management issues.

1993: Volume 1 (6 issues) ISSN: 0967-0661 (00123)
Annual Subscription 1993 £220.00 US$418.00*

### NANOSTRUCTURED MATERIALS

Principal Editors: **B. H. Kear**, **R. W. Siegel** and **T. Tsakalakos**, *USA*

The primary mission of the journal *Nanostructured Materials* is to provide a broad interdisciplinary forum for the effective dissemination of scientific and technical information on the synthesis, processing, theory, computational modeling, structure, properties, performance and applications of nanostructured materials. The following are major areas of concentration of the journal: Clusters and cluster assembled materials, structure and characterization of nanophases, synthesis and processing of nanostructured materials. *Nanostructured Materials* is published on behalf of Acta Metallurgica Inc.

1993: Volumes 2 & 3 (12 issues) ISSN: 0965-9773 (00109)
Annual Subscription 1993 US$650.00 £351.00*

Prices and proposed publication dates are subject to change without prior notice. First price quoted is definitive and applies worldwide. Asterisked price quoted is for convenience only and is subject to exchange rate fluctuation.

### STRUCTURAL ENGINEERING REVIEW

An International Journal

Editors: **Barry H. V. Topping**, *UK* and **Ronald L. Sack**, *USA*

*Structural Engineering Review* provides a focal point for innovative ideas in structural engineering. Primarily concerned with the analysis, design and construction of civil engineering structures, the interdependence of architectural and structural engineering design is highlighted. Topics covered by this journal include structural analysis, design methodology, design codes, construction and erection procedures, and the repair and maintenance of structures.

*Previously published by Chapman & Hall*

1993: Volume 5 (4 issues) ISSN: 0952-5807 (00111)
Annual Subscription 1993 £105.00 US$200.00*

## Physical Sciences

### CHAOS, SOLITONS AND FRACTALS

An Interdisciplinary Journal of Nonlinear Science

Executive Editor: **M. El Naschie**, *UK*

*Chaos, Solitons and Fractals* is an interdisciplinary research journal covering all aspects of nonlinear science including: bifurcation and singularity theory, deterministic chaos and fractals, stability theory, soliton and coherent phenomena, pattern formation, evolution and complexity theory.

1993: Volume 3 (6 issues) ISSN: 0960-0779 (00967)
Annual Subscription 1993 £320.00 US$608.00*

### EXPLORATION AND MINING GEOLOGY

The Journal of the Geological Society of the Canadian Institute of Mining, Metallurgy and Petroleum

Co-Editors: **J. F. Davies**, **H. L. Gibson** and **R. E. S. Whitehead**, *Canada*

*Exploration and Mining Geology* is an international publication dealing with mineral deposits, mining geology, geochemistry, geophysics, geomathematics and directly related environmental and Earth science studies.

1993: Volume 2 (4 issues) ISSN: 0964-1823 (00102)
Annual Subscription 1993 US160.00 £86.00*

## Life Sciences & Medicine

### ADVANCES IN NEUROIMMUNOLOGY

Editors: **G. B. Stefano** and **E. M. Smith**, *USA*

This journal publishes comprehensive reviews on significant research into the interactions between the nervous and immune systems. Areas covered include psychoneuroimmunology, immunopharmacology, immunautoregulatory mechanisms, neuroimmunomodulation and neuroimmunoendocrinology, from both basic and clinical standpoints.

*Previously published by Manchester University Press.*

1993: Volume 3 (4 issues) ISSN: 0960-5428 (00114)
Annual Subscription 1993 £165.00 US$314.00*

# FROM PERGAMON PRESS

## CELL TRANSPLANTATION

Editor: **P. Sanberg**, *USA*

This new interdisciplinary journal publishes original research and review articles on the subject of cell transplantation and its application to human diseases. It deals with a wide range of topics including physiological, medical, preclinical, tissue engineering and device-orientated aspects of transplantation of nervous system, endocrine, growth factor-secreting, bone marrow, epithelial, endothelial and genetically engineered cells among others.

1993: Volume 2 (6 issues) ISSN: 0963-6897 (00100)
Annual Subscription 1993 £172.00 US$327.00*

## EUROPEAN JOURNAL OF CANCER Part B: ORAL ONCOLOGY

Editor: **Crispian Scully**, *UK*

A wide range of malignant neoplasms involve the mouth and perioral tissues, and oral disease is a major component in many other malignancies, as a symptom of cancer chemotherapy and radiotherapy. *Oral Oncology*, the first daughter journal of *EJC* draws together the various aspects of oral and perioral oncology in one publication. The editor is Professor Crispian Scully of the Centre for the Study for Oral Disease, Bristol, UK. *EJC Part B: Oral Oncology* is supplied as part of an institutional subscription of *EJC*, and is also available separately.

1993: Volume 29B (4 issues) ISSN: 0964-1955 (00105)
Annual Subscription 1993 £125.00 US$238.00*

## ONCOLOGY RESEARCH

Incorporating *Cancer Communications*

Editor-in-Chief: **Alan C. Sartorelli**, *USA*

This new journal retains the speed of publication achieved by *Cancer Communications*, but broadens its appeal by publishing both full research papers and short communications on all aspects of the basic science, as well as reports dealing with cancer prevention and epidemiology, and clinical trials delineating effective new therapeutic regimens.

1993: Volume 5 (12 issues) ISSN: 0965-0407 (00894)
Annual Subscription 1993 £265.00 US$504.00*

## *Social & Behavioural Sciences*

## EUROPEAN MANAGEMENT JOURNAL

Editor: **Paul Stonham**, *UK*

*European Management Journal* publishes leading-edge research from academics and senior industrialists throughout Europe and the United States, enabling practitioners and management trainers to broaden their knowledge of the new operational environment. Produced on a three month publication schedule, it ensures timeliness and topicality of articles with particular relevance to European business affairs.

*Previously published by Basil Blackwell Ltd*

1993: Volume 11 (4 issues) ISSN: 0263-2373 (00115)
Annual Subscription 1993: £117.00 US$222.00*

## LOCATION SCIENCE

Editors: **Richard Church**, **John Current**, *USA* and **H. A. Eiselt**, *Canada*

The primary focus of this journal is on research directed to extending and applying the theory and modeling of location decisions. The journal will also publish papers which address the underlying processes related to locational decisions, the determination of parameters associated with location problems, and issues of implementation.

1993: Volume 1 (4 issues) ISSN: 0966-8349 (00108)
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# A dynamic quintet

*Michael Shlesinger*

**Journal of Nonlinear Science.** Editors-in-chief E. Kuznetsov and S. Wiggins. *Springer. 4/yr. $215 (institutional), $65 (personal). (Includes subscription to* Nonlinear Science Today.)

**Nonlinear Science Today.** Editors-in-chief P. Holmes and I. Stewart. *Springer. 4/yr. $29.*

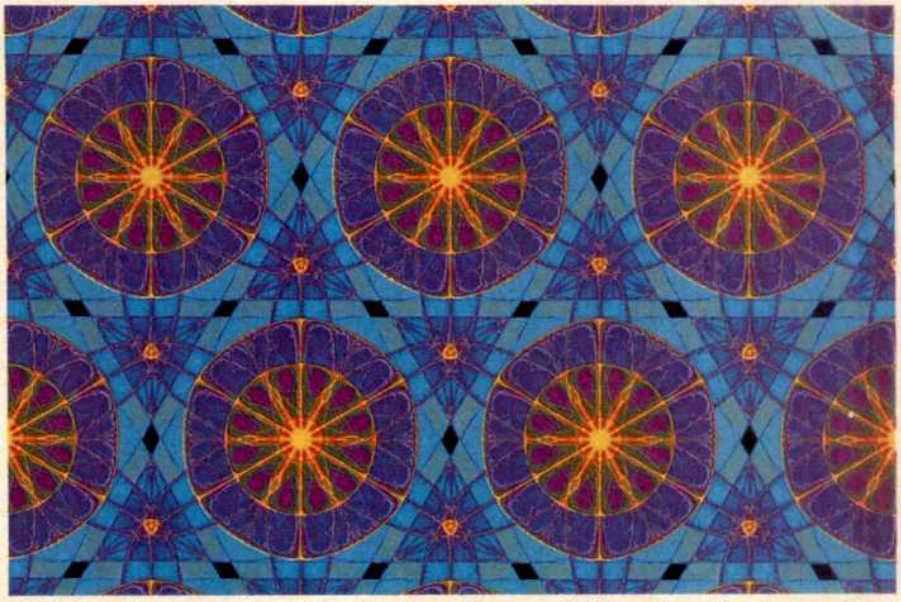
**Chaos, Solitons and Fractals: Applications in Science and Engineering.** Editor-in-chief M. S. El Naschie. *Pergamon. 6/yr. £320, $741 (institutional).*

**Chaos.** Editor-in-chief D. K. Campbell. *AIP. 4/yr. US $190, Europe and Asia $205, elsewhere $195 (institutional); $60 (personal); US $55, Europe and Asia $70, elsewhere $60 (members).*

**International Journal of Bifurcation and Chaos.** Editor L. O. Chua. *World Scientific. 6/yr. $380 (institutional), $165 (personal, and institutions/libraries from developing countries).*

As recently as the late 1970s, the best source of new work in nonlinear dynamics was Joe Ford's updated collection of preprint abstracts, which he mailed from Georgia Institute of Technology as a service to the community. This source eventually grew into the first nonlinear dynamics physics journal, *Physica D*, which was launched in 1980. Before the advent of specialized nonlinear dynamics journals, seminal works appeared in a variety of journals. The masterpieces from E. Lorenz, D. Ruelle and F. Takens, R. M. May, and M. Feigenbaum appeared in *Journal of Atmospheric Sciences, Communications in Mathematical Physics, Nature* and *Journal of Statistical Physics* respectively. Since the inaugural issue of *Physica D*, other new journals, including *Nonlinearity* (reviewed in these pages two years ago), have appeared that are devoted to nonlinear dynamics, and many of the established journals, such as *Physical Review Letters*, also attract a steady stream of papers on the subject. It is a testament to the excitement and robust health of this discipline that four new high-quality journals and a magazine have recently joined this list. Ironically, with these new journals, we have come back full circle to the pre-1980 question of where to publish a nonlinear dynamics paper.

What audiences could these journals be trying to reach? Mathematicians, physicists, chemists, engineers, Earth scientists, biologists? Almost by definition, based on the universality of nonlinear equations and phenomena, the answer must be the same for each journal — all the above. For example, period doubling, chaos, crises and strange nonchaotic attractors can be found in a wide variety of mathematical equations, physical phenomena, engineering applications and, perhaps, biological activity. Each journal wants to be the forum where different disciplines meet and interdisciplinary research based on nonlinear dynamics and fractals becomes a reality. Each journal does, however, have its own identifiable focus and flavour.



**Sundial mosaic — repeating pattern based on a hexagonal lattice. (The picture is reproduced from *Symmetry in Chaos* by M. Field and M. Golubitsky, published by Oxford University Press on 5 November and to be reviewed in a future issue of *Nature*. Price £19.95.)**

*Journal of Nonlinear Science* should attract manuscripts of a more mathematical nature. But the editors insist that its papers go beyond the specific confines of the problem at hand and paint a broader picture, with physical concepts and experimental evidence directly addressed, so at least the introduction of each paper is worth reading. Its companion publication, *Nonlinear Science Today*, is inexpensive and just plain fun to read. It is more of a magazine, with interviews, tutorial articles, reviews, opinions and announcements. It is for leisure reading and keeps one in touch with the community.

*Chaos, Solitons and Fractals*, with its emphasis on applications, should find its natural home in the engineering community. It has the opportunity to play a pivotal role in bringing this new mindset to a community that has been slow to embrace the new paradigm of chaos. The journal has also attracted some good physics papers. There is a limited use of colour (free to authors), but the camera-ready format sometimes gives the journal the appearance of lecture notes rather than an archive of scholarly articles.

*Chaos* wants to be the home for papers communicating the advances of the great Russian school of dynamics. It will even accept manuscripts written in Russian. *Chaos* can play a valuable role in joining the US and Russian communities and in decreasing the re-invention of work that has often occurred among workers in the East and West. The journal is directed towards physicists and has made an excellent start towards attaining its goals. The quality of the journal and the papers is very high.

The *International Journal of Bifurcation and Chaos* is the most handsomely produced, with its large pages and type, and it is the place to publish colour figures. The covers are breathtaking and the articles are rich with brilliant colour pictures (again free to authors). When lack of shelf space forces the clearing of my office some years from now, I shall hesitate to throw out any of these issues. Each begins with valuable tutorials and reviews, and is followed by papers and letters. It is evident that much effort has been expended to bring high-quality readable papers from a variety of scientific fields into this quarterly publication. Not just in terms of its low cost, I consider this journal the best buy.

With these five new publications (and their large editorial boards), it is clear that nonlinear dynamics has established itself as a dominant force in science. I await future issues with anticipation. □

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# Material beginnings

*Ian Walmsley*

**Nonlinear Optics: Principles, Materials, Phenomena, and Devices.** Editor Takayoshi Kobayashi. *Gordon and Breach. 4/yr. $640 (corporate); £315 (institutional); £60, $111 (personal).*

IT is some 60 years since the first prediction of a nonlinear optical effect was made, and some 30 years since such phenomena were first observed. Given the long history of this branch of physics, and the prodigious literature on the subject, it is surprising that it has taken so long for a journal named after the field to spring forth. Perhaps part of the answer is that nonlinear optics has progressed from being an endeavour primarily for the laboratory to the point where it has serious practical implications for devices devoted to information processing, notably optical communications systems. It is thus important that appropriate nonlinear media are available, and a considerable part of current research in nonlinear optics is concerned with the development of such materials.

The first few issues of *Nonlinear Optics* are, in fact, largely about materials research, as is perhaps natural for a journal that is a part of a materials science publication (it is part B of *Molecular Crystals and Liquid Crystals Science and Technology*). This emphasis is likely to be its strength, although it is the avowed aim of the journal also to provide a forum for research in principles, phenomena and devices related to nonlinear optics. These areas are, however, already well serviced by other journals such as *Journal of the Optical Society of America B, Physical Review A, Optics Letters, Optics Communications* and *Applied Physics Letters*, and it is unlikely that a new publication will usurp its more well established brethren. The question is really whether it will complement them.

On the other hand, papers on research in nonlinear optical materials have been more widely dispersed, turning up in chemistry and chemical engineering journals, as well as in journals devoted to solid-state and applied physics. So it is appropriate and timely to provide a focused repository for this information, and it is this niche that the present journal is likely to fill, provided that the content can be broadened to include a larger representation of solid-state materials, especially bulk and microstructure semiconductors.

The journal has had an auspicious beginning. The editorial representation is both geographically broad and technically comprehensive, as is appropriate for an interdisciplinary journal. The issues so far have contained mainly lengthy research articles (10 to 20 pages apiece). There is no explicit statement about the publication of comments (none has appeared, but they are not prohibited) and there are no editorial or review sections. The production quality is high, and the cost reasonably low, considering that there are no page charges. The journal in its present form provides a useful addition to an optics research library. □

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# Optical activity

*Keith Blow*

**Soviet Lightwave Communications.** Editor-in-chief A. M. Prokhorov. *IOP/Academy of Sciences of the USSR. 4/yr. £120, $215.*

A WESTERN journal devoted to scientific publications from the Eastern bloc is one that we can readily make room for. *Soviet Lightwave Communications* will publish papers in the areas of optical communications, guided wave optics and nonlinear optics. Overall, the journal has the feel — in style, quantity and content — of the *Journal of Physics* series, also published by IOP. Papers are refereed by one Western and one Eastern expert, and most seem to be published in less than six months from receipt. In his opening leading article, Prokhorov says, "we intend to publish the latest and best of Soviet fibre and integrated optics research". So far, the journal is living up to this expectation. More than half of the papers have come from the well known General Physics Institute in Moscow, but I am sure this will change as the reputation of the journal becomes established and spreads throughout Eastern Europe.

The first few issues contain several important papers that have set the standard for others to follow. For example, the first issue contains the definitive theory of long-range soliton interactions in optical fibres, a problem of relevance to transoceanic soliton communications systems. However, this is just one of many articles involving optical fibres, ranging from papers on ultrashort (less than 100 femtosecond) pulse production and propagation to papers on industrial production of fibre in the former Soviet republics. Several articles on the newer field of waveguides in highly nonlinear materials such as KTP have also appeared. All these topics are being actively pursued in the West and so we can reasonably expect the journal to become a useful source of information for all optical physicists and engineers. □

*Keith Blow is at British Telecom Development and Procurement, BT Laboratories, Martlesham Heath, Ipswich IP5 7RE, UK.*

# Images, signals and circuits

*David Holburn*

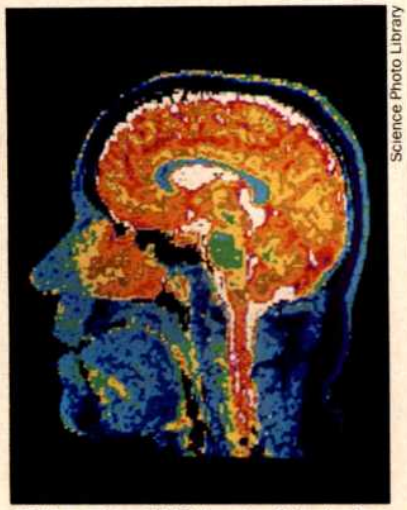
**CVGIP: Graphical Models and Image Processing.** Editors-in-chief Norman I. Badler and Rama Chellappa. *Academic. 6/yr. UK £172, elsewhere $294 (institutional).*

**CVGIP: Image Understanding.** Editor-in-chief Linda G. Shapiro. *Academic. 6/yr. UK £219, elsewhere $317 (institutional).*

**Journal of Visual Communication and Image Representation.** Editors-in-chief Yehoshua Y. Zeevia and T. Russell Hsing. *Academic. 4/yr. UK £107, elsewhere $154 (institutional); UK £56, elsewhere $81 (personal).*

TECHNOLOGICAL developments over the past 20 years have made computer graphics, computer processing of images and computer vision popular topics for investigation. Although these three activities have arisen from quite distinct roots, they overlap to a certain extent. Graphical displays are clearly important in image processing, computer vision or visual communication, while many problems in computer graphics require techniques from image processing for their solution. More recently, several new image and communication technologies have emerged, such as high-definition television, multimedia, satellite imagery and medical imaging. For many years now, *Computer Vision, Graphics and Image Processing (CVGIP)* has served admirably as a forum for workers in these fields. *CVGIP* has an excellent pedigree, running to 52 volumes, is well presented, and is essential reading for anyone with an interest in imaging or graphics.

But the pace of research eventually led the editors to consider whether the journal was trying to cover too much. The decision was taken to split *CVGIP* into two separate journals, each aimed at a different area. Two of the journals reviewed here are direct descendants, inheriting the quality of content and presentation associated with *CVGIP*; but each is also a new journal in its own




False-colour NMR image of the brain.

right, with its own objectives and editorial direction. The third journal, *Journal of Visual Communication and Image Representation*, cannot claim the same ancestry, having been created specifically to fulfil a perceived new need.

*CVGIP Graphical Models and Image Processing* concentrates on the synthesis methods or computational models that underpin computer-generated or processed imagery. Topics include image synthesis, visualization, physical models, curves and surfaces, solid models, animation, computational geometry, three-dimensional imaging, image models and transforms, enhancement, restoration, compression and mathematical approaches to image analysis.

*CVGIP Image Understanding* focuses on the topics of early vision, data structures and representations, shape, range, motion, matching and recognition, architecture and languages, and vision systems. The selection of issues provided for review included a special edition on vision based on computer-aided design. The first issue introduces a "Dialogue" section intended to include papers on controversial topics and to stimulate thought and debate. This is accompanied by responses from several people who have had an opportunity to read the papers. Additional comments are solicited from readers. I am pleased to see that the invaluable annual bibliographic survey by Azriel Rosenfeld, covering references in *Image Analysis and Computer Vision*, has been retained.

*Visual Communication and Image Representation* publishes papers on the state-of-the-art of visual communication and image representation (VCIR). This is taken to include the theory and practice of digital and analogue electronic systems, as well as biological aspects of visual communication. The review selection of issues included a special edition on VCIR in Japan.

The quality of content in each of these journals is very high. Coming as they do from the same publisher, all three have similar requirements for submission and review of manuscripts. Papers vary in length from about 10 to 30 pages, and there is plenty of variety in the subject matter. Although at first sight there might seem to be little risk of duplication of material, in the issues I saw there was some overlap, especially between *Image Understanding* and *Visual Communication and Image Representation*.

One should perhaps be wary of carping when new journals appear, for their very existence must surely indicate fertility and lively growth in the field. But to be realistic, here we have three comparatively costly journals where previously there was one, and an inexorably shrinking subscription base. I do not envy librarians the soul-searching and hard decisions they will have to make in selecting journals for their shelves.

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**Digital Signal Processing. A Review Journal.** Editors John Hershey and Rao Yarlagadda. *Academic. 4/yr. US and Canada $110, elsewhere $128 (institutional).*

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NOT many new journals these days attempt to buck the common trend towards greater and greater specialization. *Digital Signal Processing* is a refreshing exception to this rule. It describes itself as "an eclectic journal for the Digital Signal Processing community". Essentially a review journal, it aims to stress and explore creative aspects of signal processing.

The editors' declared intention is to choose for each issue a central theme, and to assemble a group of authors to write on related topics. This laudable aim is clearly a difficult one to achieve, however, and in the issues sent for review I detect a gradual shift away from this policy, although there remains a conscious attempt to offer a centrepiece article on a particular topic. In the opening issue, the chosen focus is computation; subsequent issues emphasize statistics, communications and radar, with occasional forays into such diverse topics as signal processing in the management of power.

The style of articles extends from formal research and tutorial papers to more discursive reviews and commentaries, and in this sense the journal differs markedly from more mainstream journals. As a result, we find such gems as "How I came up with the Discrete Cosine Transform", and articles that discuss application-related issues, arguably hard to find elsewhere, such as the detection of windshear using pulsed Doppler radar. But it is hard to think of a situation in which this would be the first journal one would consult in search of a reference.

The length of articles varies widely from one to eight pages. Judging by the latest references quoted by contributing authors, the delay between submission and publication seems to be no more than about six months.

A new journal must today have something unique to offer if it is to succeed. Although *Digital Signal Processing* may not attract the dyed-in-the-wool researcher, who will almost certainly have recourse to an array of heavyweight journals, it has an immediate and wide-ranging appeal. I certainly found it offers a refreshing view of a fascinating discipline, and recommend it as good value and a 'good read' for anyone involved in signal processing.

Yet another signal-processing publication? Is it really necessary? Is there really material not covered in other publications? "Yes, Yes and Yes", proclaim the editors, perhaps unsurprisingly. On balance, I think that "Yes, perhaps and sometimes" would be a fairer reply.

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**Analog Integrated Circuits and Signal Processing: An International Journal.** Editors-in-chief Mohammed Ismail, David G. Haigh and Nobuo Jujii. *Kluwer. 4/yr. DFl359, $185.50 (institutional); DFl185 (personal).*

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ANALOGUE integrated circuit design has grown rapidly over the past few years. Great progress has been made recently in bridging the gap between classical analogue circuit work and the striking advances in computer-aided design and VLSI (very-large-scale integration) processing that have resulted in the emergence of technologies (such as scalable MOS, BiCMOS and floating-gate devices and gallium arsenide) with much increased analogue potential. As well as developments in traditional areas such as amplifiers, filters and analogue interface circuits, the impact of analogue technology is now being felt in the burgeoning field of signal and information processing.

Hitherto, research papers in the analogue field have appeared in a number of widely scattered journals, and this important area of microelectronic technology has for some time been deserving of a dedicated journal to provide a forum for the scientists, engineers and educators who work within it. *Analog Integrated Circuits and Signal Processing* is thus a welcome and timely contribution. The journal publishes research and tutorial papers on the design and applications of analogue integrated circuits and signal-processing circuits and systems.

The editors have assembled a large and impressive editorial board spanning industry and academic institutions. Although most members are from the

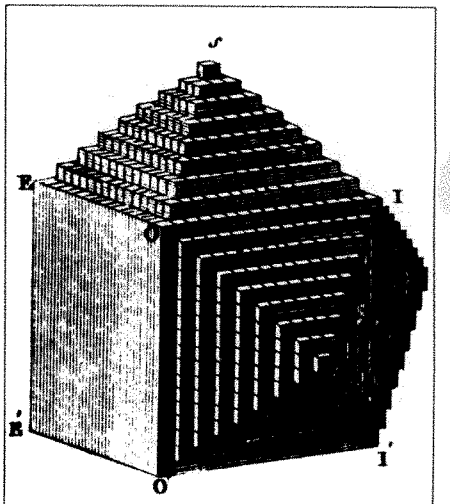
United States, there is a reasonable representation from Europe. The most serious competition to this new publication is likely to come from *IEEE Journal of Solid-State Circuits, IEEE Transactions on Circuits and Systems* and *Electronics Letters*.

Several special issues are planned, compiled under the direction of a guest editor, covering 'Analogue Design-for-Test', 'High Speed Interconnects' and 'Sensors and Sensor Signal Conditioning Circuits'.

The papers cover an impressive range of topics, and are of excellent content. Several laboratories and research groups of international renown are represented. The delay between first submission and publication — up to a year — is perhaps longer than one might wish for, but seems inevitable in view of the editors' aim to produce a journal of unassailable quality.

The journal has made a considerable impression in its first few issues, and is recommended to anyone actively involved in the field of analogue VLSI. □

*David Holburn is in the Department of Engineering, University of Cambridge, Trumpington Street, Cambridge CB2 1PZ, UK.*




**Material world: an early nineteenth-century atomistic view of crystal structure (by René Haüy), looking forwards to modern atomic clusters.**

# Thick, thin and ultra

*Robert W. Cahn*

**Advanced Composite Materials: The Official Journal of the Japan Society of Composite Materials.** Editor-in-chief H. Miyairi. *VSP. 4/yr. DM360, $234.*
**Composites Engineering.** Editor-in-chief David Hui. *Pergamon. 12/yr. £432.50 (institutional).*
**Diamond and Related Materials.** Editor-in-chief R. Messier. *Elsevier. 12/yr. DFl1101, $602.*
**Journal of Cluster Science.** Editors Richard D. Adams, David H. Russell and Boon K. Teo. *Plenum. 4/yr. US and Canada $115, elsewhere $135.*
**Journal of Inorganic and Organometallic Polymers.** Editor Martel Zeldin. *Plenum. 4/yr. US and Canada $135, elsewhere $160 (institutional); US and Canada $35, elsewhere $40 (personal).*

IF anyone still has doubts that materials science is a broad church indeed, this collection of five new journals should dispel them. In terms of subject matter, national distribution of editors and authorship, frequency and character of publication and expense, they constitute as varied a group as one could imagine. Even the appositeness of their titles varies.

*Advanced Composite Materials* is firmly Japanese, having grown out of the *Transactions of the Japan Society for Composite Materials*, which had been published in Japanese and English editions for many years. In the first few issues of the new journal, some papers from those earlier editions are reprinted, as are some papers previously published in the Japanese edition of the *Journal of the Japanese Society of Reinforced Plastics*. The president of the Japan Society for Composite Materials (in a leading article not written by the editor) makes it clear that contributions from outside Japan are welcomed, and in fact 15–20 per cent of the papers come from other Asian countries, impressive for the first year of such a journal; there is just one from the United States in the first five issues. The emphasis is entirely on mechanical properties, theory and experiment alike, including testing methods. Fabrication is said to be one of the themes, but in the early issues it is little represented. There are more foreign than Japanese members of the editorial advisory board, most of them from Europe and the United States; one presumes that their function is to increase the number of foreign papers that appear. The Japanese editorial advisers are almost exclusively from Tokyo, although papers come from all over Japan. Publication (quarterly) started in 1991, and the relatively modest subscription prices have not increased in 1992.

*Composites Engineering* is edited in the United States and has 19 foreign and 31 US editorial advisers. Frequency of publication was six times in 1991 and is to be twelve in 1993. The subscription price has increased steeply for volume 2, but is still fairly moderate. The editor goes out of his way to emphasize the speed of refereeing (three referees per paper) and editorial decision making, and of publication which, we are told, has invariably taken less than six months. In the second issue of volume 1, the names of referees (other than editorial advisers) are listed, an unusual example of editorial frankness.

The subject matter is again focused on mechanical behaviour, experimentally and theoretically conceived and at both macro and micro levels, with special emphasis on demanding environments. A good range of excellent papers has been published so far; initially almost all papers were from US authors, but other countries are now increasingly represented.

The two composites journals have certainly made a good start but they have entered a comparatively crowded market. By contrast, *Diamond and Related Materials* has created its own new niche. The declared main emphasis of this journal is on the formation, properties and applications of vapour-deposited diamond (and diamond-like) films, with only subsidiary interest in natural or pressure-generated 'massive' diamonds. Cubic boron nitride and any other materials that can effectively compete with diamond for hardness are also to be treated.

The editorial arrangements of this journal are somewhat unusual. It has one editor-in-chief and eight associate editors, well distributed geographically, but no editorial advisory board. I wonder whether this omission might be the reason for the erratic publication of the journal during its first year, and the fact that almost every paper published so far (all duly refereed) had been presented at one of the recent major conferences on diamond films. (In view of this, it is scarcely surprising that essentially all the papers up to now have been concerned with films.) Publication is announced as being monthly, but there was a seven-month gap between issue 1 (August 1991) and 2–4 (March 1992) of the first volume. Thereafter, publication seems to have been monthly, and the 'fat' issues published this year are, it seems, to compensate for the long period of silence.

The papers are generally excellent, but it is devoutly to be hoped that in future the incidence of conference papers will diminish. It may be necessaary to establish an advisory board to ensure that this happens. Provided that it does, this journal clearly fills an essential niche and should have an assured future. There can be no doubt of the scientific interest and the considerable industrial

potential of this relatively new field of thin-film diamond and closely related topics.

For a materials scientist, the quarterly *Journal of Cluster Science* is a disappointment. The study of very small atomic clusters, both in the free state and in 'cluster-assembled' form (in which form they are nowadays termed 'nanostructured materials'), has grown by leaps and bounds in the past few years; physicists, chemists and materials scientists have been equally involved. Thus, in 1991 there was an international symposium in the United States on the "physics and chemistry of finite systems: from clusters to crystals" ('finite systems' is only the latest of the curious terms that abound in this broad field), while in September 1992 there was a major international conference in Mexico devoted to nanostructured (assembled) materials, which in spite of its name also included attention to free clusters. By contrast, the new journal is virtually exclusively chemical in its ambit: all three editors are chemists, and because I do not recognize the names of any of the editorial advisers, I presume that they are also all chemists.

Almost all of the papers appearing in the first year (starting 1990) are purely chemical, dealing with synthesis, structure analysis and reactivity of clusters (with good variety within this limitation), but there are a few mathematical papers about topology of clusters and one on the electronic structure of small lithium clusters. I am afraid that this journal, as it is now, will (despite its low cost) be of only minimal interest to the broader community of researchers concerned with clusters and cluster-assembled materials. It is probable that a newly established Pergamon journal, *Nanostructured Materials*, which is likely to be reviewed in these columns next year, will better fill the needs of the broad community.

The journal has one very peculiar feature: issue 2 of volume 2 (June 1991) contains a paper labelled as having been received on 17 June 1991, while issue 3 of volume 2 (September 1991) contains a paper labelled as having been received on 7 November 1991. The speed of refereeing for this journal must exceed the speed of light. More seriously, the editors might care to consider whether a broadening of the journal's scope would enhance the inflow of manuscripts, as well as the saleability of the journal.

The quarterly *Journal of Inorganic and Organometallic Polymers* is again chemical in flavour. It is focused strongly on silicon-containing polymers such as polysilanes, polysiloxanes, polysiliazanes and polycarbosilanes; the papers in the first few issues show a good range covering synthesis (both of monomeric precursors and of the polymers themselves), fibre production, radiation-curing and degradation of these polymers, but there is relatively little emphasis on properties and applications. The journal is cheap and the subscription price has not been increased for volume 2, although more pages are being offered.

As far as I can ascertain from the copies I have seen, all papers published so far (1991–92), both reviews and research papers, stem from conferences (they seem to have been peer reviewed). I cannot help thinking that to run a new journal exclusively in this way for more than a year almost amounts to false pretences. A scientific periodical, especially if it aspires to archival status, should contain mostly freely submitted papers. With that out of the way, I can welcome the journal as filling a distinct need; again, however, some broadening of scope beyond chemistry alone would be welcome. □

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# Chemical crosslinks

*Leslie Crombie*

**Mendeleev Communications: Preliminary Accounts of Important New Work in Chemistry from Russia, Other States of the Commonwealth of Independent States, and Elsewhere.** Editors O. M. Nefedov and H. M. Frey. *Academy of Russian Sciences/Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 4WF, UK. 6/yr. Europe £140, US $280, Canada £144, elsewhere £140.*

SINCE the Second World War, scientific contacts in chemistry between the West and the former Soviet Union have been of a rather limited nature. Conference organizers have been dismayed by the sudden refusal to allow an invited Soviet speaker to come, or by the sudden appearance of a different, uninvited and less suitable scientist who was presumably more politically acceptable. This, of course, has been merely the tip of an iceberg. My following of Soviet organic chemistry has been a faithful perusal of the text and graphical abstracts from the Soviet journals included in *Index Chemicus* over more than 25 years, and I have found it difficult to extract the really good work that is undoubtedly being done from much mediocrity. The Soviet Union suffered from a shortage of equipment during that period and, importantly I think, failed to develop chemical supply houses of anything like the quality and range familiar in the West. But the problems have been deeper than this. There seems to have been a damaging political control by scientifically inferior party men who choose poor problems and worked to narrow horizons. Fortunately, all this belongs to the past and we can look forward to a much brighter future, for which this new journal is a harbinger.

I regard the far-sighted idea behind *Mendeleev Communications* as highly commendable, and of great credit both to the former USSR Academy of Sciences and to the Royal Society of Chemistry. Published in English, it contains short papers of a kind familiar in the *Journal of the Chemical Society and Chemical Communications*. Indeed, except for the fact that almost all its papers originate from former Soviet territories, its size and format are identical to the latter. The best of the chemistry produced in these countries is now easily accessible in English, and there is no excuse for overlooking such important contributions. Also, the contributors stand a better chance of gaining international recognition for their work.

Although the first issue is dated February 1991, the journal is still in its first volume of eight issues and its rate of publication has been quarterly. I surveyed four issues containing 89 papers and found the subdisciplines of chemistry to be represented as follows: organic 53 per cent, physical 29 per cent and inorganic 18 per cent. As a whole, the quality of the papers, while good and interesting, did not seem to me to be of quite the general excellence of *Chemical Communications*, but on reflection this is hardly surprising, for the latter covers the world's chemistry and it can afford to be highly selective. In the first issue of *Mendeleev Communications*, it was indicated that the time from receipt of a manuscript to its publication would be three months — reasonable for a communications journal. In fact publication is taking much longer, perhaps because both Russian and British referees are involved. Despite the double refereeing, improvement in the speed of publication is desirable.

The journal is a source of useful information on meetings in former Soviet territories, and its "Academy News" reflects some of the deep-seated changes that have taken place over the past couple of years. Apart from meetings and the proceedings of the Academy, there is also useful information on chemistry teaching in Russia.

Before the coming of the Communist era, Russia had well known schools of chemistry, important in history, with

Mendeleev outstanding among its scientific figures of world status. If, as I think it does, *Mendeleev Communications* helps to restore that contribution to international chemistry, its promoters may indeed rest well satisfied. □

*Leslie Crombie is in the Department of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, UK.*

# Drug design

*Keith James*

**Bioorganic and Medicinal Chemistry Letters.** Editor-in-chief D. L. Boger. *Pergamon. 12/yr. £378 (institutional), £65 (personal).*
**Medicinal Chemistry Research.** Editors Richard A. Glennon and Alfred Burger. *Birkhäuser. 9/yr. US and Canada $275, elsewhere $295 (institutional); US and Canada $80, elsewhere $95 (personal).*

MEDICINAL chemists are frontline troops in the battle to discover new therapies for many of the diseases of our day. As both architects and construction engineers at the molecular level, they constantly refute Solomon's assertion that 'there is nothing new under the Sun' by designing and synthesizing novel, biologically active agents. The appearance of two new rapid-communication journals, in which these chemists can describe and appreciate drug-design principles and synthetic achievements, is therefore welcome.

*Bioorganic and Medicinal Chemistry Letters* is here to stay. Its title accurately reflects its content and style, which is the key to its successful impact. Out of the Pergamon stable, this monthly publication has the look and feel of *Tetrahedron Letters*, essential reading for all synthetic organic chemists, from among whose numbers almost all medicinal chemists come. It provides authors with the option of a two-, four- or six-page camera-ready format, and readers with snappy graphical abstracts with which to scan its contents. Its 'biorganic' description ensures that, in addition to catering for the pharmaceutical arena, it attracts authors and readers from the academic community, who are increasingly drawn to the conceptual challenges of understanding the relationship between structure and bioactivity. A further welcome carry-over from *Tetrahedron* are the "Symposia-in-Print", thematic editions addressing the latest developments in areas of high interest.

Although publication in *Journal of Medicinal Chemistry* is likely to remain an aspiration for most medicinal chemists, that journal's limited scope for rapid communications and delay on full papers provides the niche that *Bioorganic and Medicinal Chemistry Letters* now fills. Indeed, for target-oriented medicinal chemists, whose *raison d'être* is not primarily to publish but to discover drugs, pithy, easily read papers, readily generated with today's desktop publishing tools, are ideal.

*Medicinal Chemistry Research* is a parallel, rapid-publication vehicle aimed at medicinal chemists, who are not, however, as familiar with it as they are with its competitor, *Bioorganic and Medicinal Chemistry Letters*. Although the stated scope of the two journals is similar, and the material within *Medicinal Chemistry Research* of interest, neither the range of topics covered in its papers nor the background of the authors is as diverse as those in *Bioorganic and Medicinal Chemistry Letters*. Also, the greater average length and more heterogeneous style of papers in *Medicinal Chemistry Research* do not help to distinguish it from the many other journals pressing for attention on library shelves. More key papers from the very best industrial and academic groups are needed to establish it as essential reading.

*Bioorganic and Medicinal Chemistry Letters* is at the high end of the price range for chemistry publications of this frequency, and *Medicinal Chemistry Research* at the low end. In the event of a budget conflict, go for the former. □

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# Transducers transformed

*Richard Compton*

**Sensors and Actuators B: Chemical.** Editor K. Cammann; editor-in-chief S. Middlehoek. *Elsevier. 12/yr. SFr1,620.*

IN little more than a decade, the field of analytical chemistry has metamorphosed from near obscurity and introspective dullness into a lively, vigorous field with a plethora of new and authentic real-world applications, and full of the stimulation of genuine interdisciplinary interaction. The perceived targets (but not the jargon) remain the same — the research and development of sensor elements to transform and transduce chemical signals into information about the chemical composition of the sample analysed. *Sensors and Actuators B* seeks papers concerned with fundamental aspects; sensor materials, effects, systems, devices including interface electronics; and the development of new chemical sensors and intelligent analytical probes for general applications in gaseous, liquid and solid samples in such areas as on-line monitoring in process control, environmental status sensing and life sciences. Typical examples of what this means in practice include chemically sensitive and ion-selective field-effect transistors, integrated optics and fibre-optical devices, remote sensing, chemically modified microelectrodes, thermal transducers, semiconducting gas sensors, Langmuir-Blodgett films, humidity sensors, not to mention electronic noses, raindrop sensors and much more. Exciting and fascinating stuff but certainly not what, say, *The Analyst* was (or is) made of.

*Sensors and Actuators* was originally born in 1981, expanded rapidly with the subject and fell apart under its own weight in 1990 into two sibling journals, *A: Physical* and *B: Chemical*; the two parts now jointly comprise no fewer than 27 issues per year. The product is very characteristically Elsevier; it is beautifully, carefully and clearly reproduced on high-quality paper, impressively free of errors, but unhappily expensive. Papers appear to be published with tolerable speed (six to eight months seems typical) and without resort to camera-ready submissions, although considerably greater delay accompanies those (not infrequent) issues exclusively devoted to recording proceedings of rather arbitrarily selected conferences and workshops.

Expensive new journals have to meet a clear need and to attract important and authoritative contributions rapidly if they are to succeed. *Sensors and Actuators B* has done both. First, its defined area lies neatly between the fundamental science covered in regular chemistry and materials journals on the one hand and the technology–engineering applications literature on the other, so providing an outlet for papers that might otherwise have been uncomfortably accommodated elsewhere and hence a forum for a re-emergent discipline. Second, the papers are generally of impressively good quality and often of surprisingly wide interest, both of which suggest effective editorial influence. *Sensors and Actuators B* reflects accurately the activities and interests of born-again analytical chemists and will be essential reading for all in the field. □

*Richard Compton is in the Physical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ, UK.*

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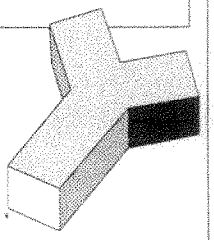

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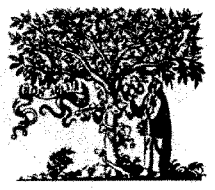

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H. J. Melosh

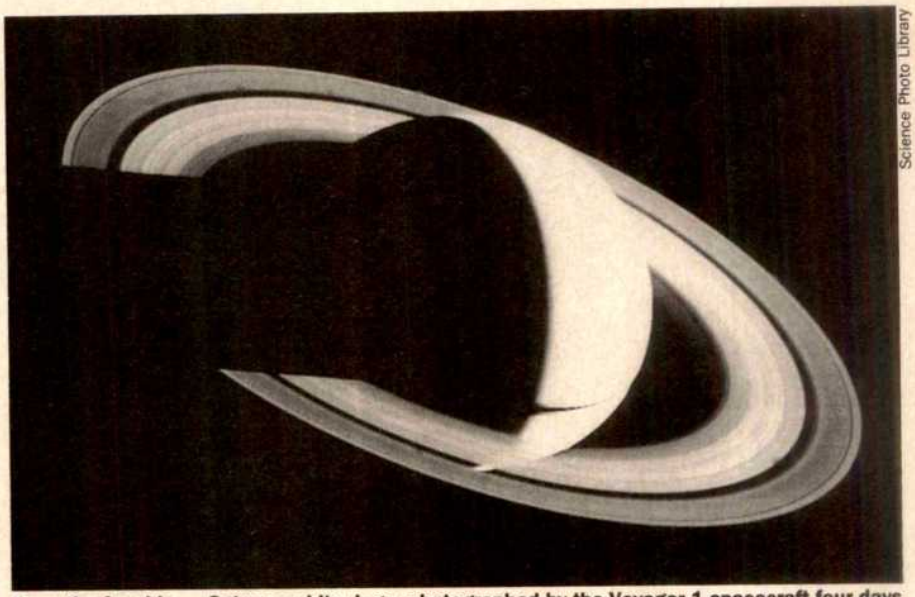
**Journal of Geophysical Research – Planets.** Editor Clark R. Chapman. *American Geophysical Union. 12/yr. $285 (institutional), $42 (members).*

OVER the past few decades, 'planetary science', a new interdisciplinary field, has crystallized from the traditional fields of geology, geophysics, geochemistry and astronomy, among others. The main concern of this new research area is the current state, origin and evolution of planetary systems, principally our own. Growth of the field has been fuelled largely by the space-exploration programmes of the United States and the former Soviet Union (with notable contributions from a few other nations). Many traditional geology departments have added the word 'planetary' to their names, and many recent texts in both astronomy and geology contain substantial discussions of the other planets in the Solar System, emphasizing comparisons between the Earth and these other planets.

One of the sure signs of the emergence of a new field is the creation of one or more journals devoted to it. Planetary science has been served for decades by journals such as *Icarus, Meteoritics* and such interdisciplinary journals as *Earth and Planetary Science Letters* and *Physics of the Earth and Planetary Interiors*. Until recently, however, the American Geophysical Union's prestigious *Journal of Geophysical Research* (*JGR*) has not had a special planetary-science division, although it has carried many important papers on planetary science in its main divisions of *Solid Earth, Space* and *Atmosphere Sciences*. (Interest in planets by the other division, *Ocean Science*, may have to await detailed study of the putative oceans on Titan.) An attempt to unify the peculiarly planetary contributions to *JGR* was made with an ancillary publication, *Planetology Papers,* which contained reprints of planetary-science papers published in the other divisions of *JGR*. In August 1991, a new formal division of *JGR, Planets* (often referred to as 'JGR Green', from the colour of its cover) was launched under the editorship of C. R. Chapman as a journal devoted explicitly to planetary science.

From the beginning, it was clear that there would be difficulty drawing boundaries between 'planetary science' and the more traditional divisions of *JGR*. For example, in the inaugural issue, Chapman wrote that papers on cometary nuclei and comas should appear in *Planets*, but that papers on cometary ion tails should not. Pity the hapless author who should submit a manuscript that deals with both comas and ion tails. All manuscripts on impact cratering, including those on the Cretaceous/Tertiary boundary, are to be handled by *Planets*, in spite of the exceptionally cross-disciplinary nature of this subject. In one of my own favourite areas of research, it is not unusual for papers on some aspect of planetary tectonics to have important implications for terrestrial tectonics, or vice versa. Although I have now submitted a paper to *JGR – Planets* (on rock fragmentation by impacts, a subject with close ties to other traditional areas of solid Earth science), the decision to send it there as opposed to other journals was not made without considerable anguish. My own experience and frustration has been echoed by many colleagues who are in the habit of studying both the other planets and the Earth. Although the pages of *JGR – Planets* are numbered consecutively with the other divisions of *JGR*, many individual subscribers to the old *JGR – Solid Earth* will not routinely see papers that may bear importantly on their own research. So the creation of *JGR – Planets*, far from filling an existing gap, has created one. It draws an artificial distinction between research exclusively on the Earth and that on the other planets, leaving no natural place to send papers that treat the Earth as one of the other planets. I see this as a backward step in the current trend of comparative thinking in Earth science.

On other matters, the quality of the papers that have appeared so far is rather mixed. Many of them conform fully to the high standard that distinguishes other divisions of *JGR*, but a large number do not. The editor is breaking new ground for *JGR* by publishing several nontraditional manuscripts, such as papers on historical matters or papers analysing the capabilities of hypothetical instruments. An entire issue is devoted to the Mars Observer spacecraft, a mission which was launched only last week. The editor's intention to publish high-quality papers covering any area of interest to planetary scientists is laudable, but it seems unlikely that papers that do not report scientific achievements will boost scientific interest in the journal.



Science Photo Library

**From the far side — Saturn and its rings, photographed by the Voyager 1 spacecraft four days after it had flown past the planet.**

*JGR – Planets* has clearly had a difficult time getting off the ground: the initial issue contained nine papers and 130 pages, mostly inherited from *JGR – Solid Earth*. This good start fell to a low eight months later, when the journal appeared as a pamphlet containing three papers and 38 pages. The size of the journal is now increasing again, and there are promises of some fatter tomes such as the special issue on Magellan and the forthcoming one on Magma Oceans. The range of topics covered has tended to vary widely, with swings from issues dominated by planetary tectonics to issues dealing mainly with atmospheric physics. To me, it seems that this range is too broad: very few people interested in, say, ionospheric physics, will be inclined to read papers dealing with the spacing of wrinkle ridges on Mars.

The production quality of the journal is excellent, conforming to the other *JGR* journals. Printed on glossy archival paper, the type is easy to read and half-

tones are clear. Copy editing is of a high standard, despite the use of much camera-ready copy prepared by authors. On the other hand, there have been long delays in the editorial processing of manuscripts. Many authors (including myself) have experienced delays of around six months between submitting manuscripts and receiving referees' reports. Many authors of the Magellan 'six month' reports have been particular frustrated by these delays. The editor has described the reasons for the delays in a recent leading article and promises to improve turnaround times in the future.

Pricewise, *JGR – Planets* is a bargain, especially for members of the American Geophysical Union. Whether it will succeed as a journal independently of the other *JGR* divisions, however, is not yet clear. At the moment, the number of papers in any one issue (excluding the special issues) is smaller than most of the topical groupings now used in *JGR – Solid Earth* (in which papers are divided into groups dealing with a common theme, such as seismology and mineral physics). And it is not obvious why papers with more of a planetary orientation could not be handled in a similar way. Such an arrangement would emphasize the role of planetary studies as an extension of traditional geophysics rather than a disjointed endeavour. □

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# Redressing the balance

*Michael Rowan-Robinson*

**Astronomical and Astrophysical Transactions: The Journal of the Soviet Astronomical Society.** Editor-in-chief Nikolai G. Bochkarev. *Gordon and Breach. 4/yr. $245, DFl550 (institutional); $445, DFl858 (corporate); $87, £59 (personal).*

THE emergence of a new Soviet Astronomical Society in 1990, and of its journal *Astronomical and Astrophysical Transactions* in 1991, is a development that will be welcomed throughout the astronomical community. The leading article in the first issue of this journal points out that: "The formation of our society is in a definite sense a re-creation of professional astronomical societies. These societies operated in Russia from the end of the last century until the beginning of the 1930's, when the majority of society organisations in the USSR were forced to cease their activity."

An independent astronomical society will clearly be of great importance for the development of astronomy in Russia and in the other republics of the former Soviet Union (like the new society, I will use Soviet to refer to the republics of the former Soviet Union). It is excellent that the society is showing its determination to thrust itself into the international limelight by publishing in English. The insularity of much Soviet astronomy has in the past been a real obstacle to progress. Too many papers were published in Soviet journals that showed little effort to become acquainted with the international literature. This meant that despite many outstanding individuals and some good institutes and observatories, the impact of Soviet astronomy has been rather limited since the Second World War. The dominance of some brilliant theorists has perhaps encouraged a cavalier attitude to observations. It is in this area of observational astronomy that most effort will be needed to put Soviet astronomy on a level-footing with European astronomy.

*Astronomical and Astrophysical Transactions* intends to publish normal-length and short papers, review articles, short items reporting current research and book reviews, as well as longer papers in the form of supplements. The first volume consists of four slim parts each of about 80 pages. The price per volume is therefore high. The quality of the papers in the first volume is very variable, the main problem being that there are too many short papers with very little detail. There is a good review by V. I. Slysh of radio supernovae and particle acceleration and an important article by N. A. Tikhonov *et al.* on the distances to three nearby dwarf galaxies in the M81 group. The interesting review by Yu. N. Pariskij on radioastronomy in the next century would have benefited from more detail, more explanation and some references. As it is, it will be fully appreciated only by those already familiar with Soviet radioastronomy.

It will be vital for Soviet astronomy that this new journal succeeds. Whether it does will depend on whether the editors are willing to give adequate space for work of real quality and to demand the highest standards of referencing and explanation. With its beautiful production, *Astronomical and Astrophysical Transactions* is off to a good start. □

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# Mutual aid

*Timothy O'Riordan*

**Journal of Environmental and Resource Economics.** Editors-in-chief J. B. Opschoor, H. Folmer and H. M. A. Jansen. *Kluwer. 6/yr. DFl488, $305 (institutional); DFl270, $169 (personal); DFl195, $122 (members).*

ENVIRONMENTAL economics is slowly coming of age, after a long period of being an odd intellectual fetus in the not-so-nurturing womb of mainstream economics. The basic principles of traditional environmental economics rest on the welfare theories of the early part of this century. In many respects there is nothing theoretically radical about much of what this subgroup of the profession has to offer. Welfare functions, production functions, indifference curves, discount rates and willingness-to-pay measures abound in a thoroughly unstartling fashion. So why has environmental economics taken so long to have been born?

One reason is that there have been few champions in a sufficiently powerful position to move the profession. Another is the internal debate between extending existing economic theory and creating a whole new model. A third is the failure, until recently, to attract the attention of policy-makers, especially those in finance and developmental bureaus, simply because environmental damage was the Cinderella of economic-growth theories and practice. A fourth is that there were few mainstream journals that catered for this aspect of research, particularly as it applied to 'real world problem solving'.

This lively new journal is one of three now offering a haven for the burgeoning band of environmental economists. The other two are thoroughly complementary. The *Journal of Environmental Economics and Management*, despite its name, is essentially geared to the theoretical end of the economics spectrum, whereas *Ecological Economics* (reviewed in last year's supplement) focuses on the interface between valuation and environmental functioning of ecosystem processes. The new journal specifically seeks to bridge the gap between economic theory and applied case studies as they relate both to management and to policy. The coverage includes not only evaluation of non-market goods and bads, the hallmark of this area of research, but, equally importantly, the economic consequences of policies, for example carbon taxes or waste-recycling regulatory measures.

The early issues reflect the interests of the senior editors, who are distinguished agents in the use of economic instru-

ments or policy, and in the environmental-sustainability indicators in resource use, especially agriculture. Sadly, to my mind, there is still too much theory and too many abstract equations, at the expence of gritty policy analysis. But one or two articles, for example on what to do when respondents to willingness-to-pay questions offer an infinite price, do stimulate the intellectual adrenalin. Hopefully, as the journal matures, more will follow. □

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# Time and space

*A. M. Mannion*

**The Holocene: An Interdisciplinary Journal Focusing on Recent Environmental Change.** Editor John A. Matthews. *Edward Arnold. 3/yr. US and Canada $225, UK £125, elsewhere £115 (institutional); US and Canada $85, UK £49.50, elsewhere £55 (personal).*

THE *Holocene* has been launched at a most opportune time: the cognizance of global environmental change is growing rapidly and environmental scientists are establishing hitherto unrecognized links between the various Earth surface processes. In geological terms, the Holocene consists of the past 10,000 years of the Earth's history and represents a period of change unprecedented in the Earth's earlier history, change due, not least, to the emergence of modern humans. A journal that includes interdisciplinary representation, international coverage and methodological advances is thus to be welcomed.

**Reversal of fortune — a glacier which loomed over the French alpine village of Argentière in the mid-nineteenth century. . .**

The format is somewhat different from that of most journals of an environmental character. Each part consists of four sectors: research papers of around 6,000 words, which are full-blown contributions to original research; research reports of around 3,000 words, which are shorter synopses of new findings or advances in methodology; forum articles of around 3,000 words, which are brief discourses on Holocene issues and may be speculative and provoke discussion; and book reviews.

The international tenor of the journal is reflected in its editorial advisory board and by the range of papers in the first volume. The United Kingdom is well represented, but there are papers on North America, China, the Middle East and South America; Australia is not represented, but it is early days for a journal with such a broad remit. The multidisciplinary aspect of the journal is also reflected in the contents of the first volume. Physical, chemical and biological evidence, either alone or in combination, for environmental change is presented. The forum articles are particularly stimulating and provide an arena for debate that is often lacking in related journals.

The A4 format of the journal is attractive and the quality of text and diagrams is very good, as are the subscription rates. The period between the initial receipt of manuscripts and publication varies between five and nine months.

In view of the quality that has been demonstrated so far, and the growing realization that the prediction of future environmental change is to a large extent dependent on understanding past environmental change, *The Holocene* deserves to succeed. □

**. . . has now retreated far up the valley. (Contemporary engraving, top, and photograph, 1966, both taken from *Global Warming* by A. Revkin, American Museum of Natural History Environmental Defense Fund, £20.)**

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# Out of focus

*Michael Grubb*

**Global Environmental Change: Human and Policy Dimensions.** Editor J. K. Mitchell. *Butterworth-Heinemann. 4/yr. Europe £110, elsewhere £115.*

THE first issue of this journal describes its role as being "to balance the discourse on global change" by giving special attention to the contributions of "social and behavioural science, policy science and environmental management". In this endeavour it shows every sign of becoming a success.

Papers are generally about 6,000–9,000 words long, well laid out with good use of footnotes. Some of the papers could have been shorter, but in general they present new and relevant information and analysis. No submission dates are given, but the content of the papers suggests reasonably rapid publication. There are no letters or correspondence, but shorter "Viewpoint" articles allow serious debate, the breadth being illustrated by an excellent exchange between one author arguing that climate change will be generally beneficial and another presenting more critical and cautious views. Reports on recent institutional developments, book reviews (rather thin on the ground so far), conference reports and a regular monitoring of relevant activities at the United Nations University provide useful background for any researcher in the area.

Maintaining editorial direction for an interdisciplinary journal is not easy, and the focus of this journal is still somewhat unclear. To my mind, there is a slight preponderance of theoretical social-science papers, with rather fewer on either management science or practical policy analysis and experience; there is also a striking lack of papers addressing economic issues. The journal thus overlaps sporadically with some other journals, notably its sister publications *Natural Resources Forum* and *Environmental Values*, as well as with more specialized publications such as *Climatic Change*.

The early issues are uncomfortably dominated by US authors, but the most recent show a broader reach, although there is still a worrying absence of papers from developing countries. Without any special concessions, the journal will almost certainly be beyond the reach of libraries in these countries, whose future path of development is so crucial to the issues that it addresses. □

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# Energy plants

*W. Bernard Betts*

**Biomass and Bioenergy.** Editors J. Coombs, D. O. Hall, R. P. Overend and W. H. Smith. *Pergamon. 12/yr. £310.*

TODAY'S sources of energy and chemicals centre largely on nonrenewable materials such as coal, natural gas and especially oil. But diminishing reserves of these materials, uncertainties of supply and cost, and worry about carbon dioxide imbalances and pollution caused by the use of these materials have stimulated great interest in renewable resources such as lignocellulosic materials (for example, wood, straw and derivatives) and other plant residues. These are available through natural biomass turnover, farming and forestry, and from municipal wastes and industrial processing.

*Biomass and Bioenergy* is a new international journal, first published in 1991, which focuses on biomass production and use. The various key areas covered are categorized as biomass (sources, energy and crop production processors), genetic improvement and composition, biological residues (agricultural, forestry, industrial and municipal wastes), bioenergy processes (fermentations, thermochemical conversions, liquid and gaseous fuels, and petrochemical substitutes) and bioenergy use (direct combustion, gasification, electricity production, chemical processes and by-product remediation). Environmental, management and economic aspects of these areas are included in the scope of the journal.

The journal accepts original research-and-development articles, book reviews, conference reports, details of forthcoming meetings, letters to the editor and topics of special interest. Its editorial team consists of 28 international members, mainly from the United States and Europe. There is minor representation of the Middle East, the Far East, India and Africa but, surprisingly, not of South America. Papers so far, however, have had a truly international breadth supporting the editors' recognition of the importance of biomass and bioenergy in both the developed and developing world.

The style and layout of the journal are traditional rather than old-fashioned and its applied stance is immediately obvious from the front cover photographs, which will continue to feature biomass and bioenergy projects. The contents are conveniently listed on the back cover and forthcoming articles detailed inside. Presentation is clear, with a large double-column format, and instructions stood. Speed of publication seems to vary somewhat, although most papers have been accepted within four mounths of receipt.

*Biomass and Bioenergy* competes with a wealth of quality journals, particularly the established microbiology, biotechnology, chemical and analytical journals, and it will have to maintain its speed of publication and quality of articles if it is to rival them. One might expect its appealing title automatically to attract workers in the field, but with such a wide scope the journal may have difficulty preserving the standards set in the first few issues. Careful editorial management is required. □

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# Thinking deep

*John Fox*

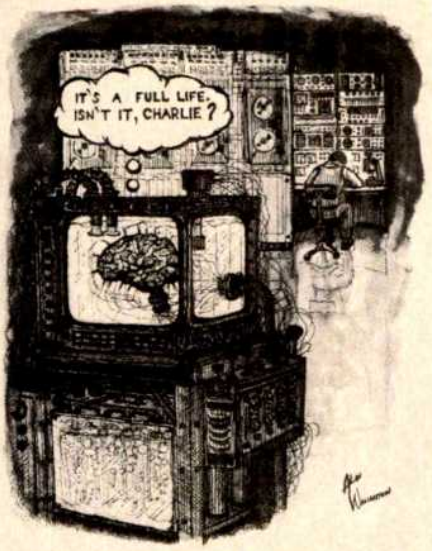
**Minds and Machines: Journal for Artificial Intelligence, Philosophy, and Cognitive Science.** Editor James H. Fetzer. *Kluwer. 4/yr. DFl360 (institutional), DFl90 (personal/members).*

ABOUT 20 years ago, I found myself in discussion with a prominent epistemologist. Being rather keen on artificial intelligence (AI), I asked him whether he found developments in that field of any interest. He was perfectly courteous, but left me in no doubt that AI was irrelevant to serious philosophical thought. I was astonished. In its distinctive way, AI was deeply concerned with mind, perception, purpose, knowledge and all that stuff you expect to find in philosophical discussions.

Admittedly the subject was still finding its feet just then. It had a tendency to ad hoc methods of enquiry (its methodology was sometimes referred to as experimental programming), and a blithe disregard for what investigators in other disciplines might have to offer.

However, AI has changed considerably in the period since that conversation. Most strikingly, the dominant paradigm now seems to be mathematical rather than experimental. AI routinely applies formal frameworks to its many areas of enquiry; language processing, machine vision, reasoning, decision making, learning, the representation of knowledge — indeed most of its major areas of research.

This may explain why I now find myself reviewing the journal *Minds and Machines*, whose editorial statement in volume 1, number 1 indicates that the journal's focus is "philosophical aspects




**Cartoon taken from *The Age of Intelligent Machines* edited by R. Kurzweil (MIT Press, 1992, $22.50 (pbk)).**

of computer science, of artificial intelligence, and of cognitive science".

Because to my knowledge there are about 20 journals directly serving the AI research community (and probably as many again dealing with AI applications), you can guess my initial reaction. On reading the editor's preamble further I found: "This journal is published in the belief that, here as elsewhere, rational criticism is our most reliable tool in the unending search for truth." As one who is trained in a natural-science approach to truth seeking, though who has adopted computing as his working technique, I did not anticipate much value from the navel gazing that I expected to follow. In fact I was very impressed. I found many interesting and stimulating papers, the quality of which is uniformly excellent. As a source of careful reflection and scholarship, the journal cannot be faulted.

However, I am not quite sure who it is aimed at, nor whether it has a distinct market. Formal "logics of arguments" and "default reasoning"; thoughtful pieces on "the many uses of belief in AI", and reflections on what neural networks actually do, can be found in any number of established AI journals, and the distinctive contribution of philosophical analysis is not immediately obvious. Nor do I find the balance that I would expect in a philosophy journal. Logic and inference are very well served, but perception, consciousness, purpose and so on are hardly in evidence. AI scientists sometimes have an amateurish go at such topics, but philosophers have thought about them deeply and could make a real contribution.

I hope *Minds and Machines* makes it, but I think it needs to broaden its philosophical base and carve out a distinct methodological niche for itself. Otherwise it will be just another AI journal and, given the competition, perhaps an unsuccessful one. □

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# Neurocomputing

*John A. Hertz*

**Network: Computation in Neural Systems.** Honorary editor Daniel J. Amit. *IOP. 4/yr. US and Canada $376, elsewhere £175 (institutional); US and Canada $71, UK £30, elsewhere £33 (personal).*

THE renaissance of research on neural networks in the past decade has spawned a welter of new journals. Only a few of them will survive the next decade: *Network* stands a good chance of being one of these.

Like the other journals, *Network* aims for a broad coverage of the field. And like most of them, it ends up with a territory within the field and a particular flavour of its own, both reflecting the influence of its honorary editor, Daniel Amit. A theoretical physicist by background, Amit was among the first to apply the powerful techniques of modern statistical mechanics to formal neural-network problems. He then turned to doing this style of modelling in a real neurobiological setting. It is here that *Network* is strongest — theoretical papers of a high level of mathematical sophistication, confronting real data and trying to do an honest job of modelling neurobiological systems and phenomena. Of course, the jury is still out and in all probability will be out for many years on whether this enterprise succeeds, but if you want to learn about how far we've got, *Network* is the place to read about it.

The spectrum of papers is of course broader than this focal area of the intersection of statistical mechanics and neurobiology. The editors have tried to enhance the breadth further through a section in which abstracts of selected papers in other journals are reproduced, together with a paragraph or two of comments by members of the editorial board or their colleagues. It is admittedly a biased, but nevertheless a valuable, view of the field that one gets through this window, a judgement that also applies to the book reviews.

In addition to papers of normal length, *Network* solicits and publishes a few "Letters to the Editor" (rapid publication) and review articles. The letters will probably have a difficult time competing with those in *Neural Computation*, but the review articles may prove to be an especially important service.

*Network* is published by the Institute of Physics in the United Kingdom. I hope this does not mean that that researchers at the neurobiological end of the field will tend to overlook it. With a bargain personal subscription rate, the journal will certainly not be beyond their reach. □

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# Beyond numeracy

*Tony Barnard*

**Mathematics Review.** Editors David Mond, Ian Stewart and David Tall. *Philip Allan. 4/vol. £16.50 (£7.95 for students at educational establishments with an institutional order).*

**Quantum: The Student Magazine of Math and Science.** Founding editors Yuri Ossipyan, Sheldon Lee Glashow and William P. Thurston; physics editor L. D. Kirkpatrick; mathematics editor M. E. Saul. *National Science Teachers Association / Springer. 6/yr. $28 (institutional), $18 (personal), $14 (student).*

THE mathematical diet of school pupils, both in content and presentation, is always influenced and constrained by national examinations, and pupils are sometimes not able to engage in as many exciting areas of mathematics or reflect on as many underlying central themes as might be desired. Complementary sources of mathematical nutrition are therefore warmly welcomed, and both *Mathematics Review* and *Quantum* are valuable morsels at the school–university interface.

*Mathematics Review* is aimed specifically at helping A-level and H-level mathematics students with their coursework and examination preparation in a broad mathematical context free of syllabus-type constraints. It is full of lively articles that both explain essential mathematical concepts and illustrate how they apply to everyday life, and is sprinkled with thoughts on heuristic and philosophical aspects. There is a good balance in the choice of material and it is well matched to the intended readership. Regular features include an examiner's eye view of examination questions and solutions, an imaginative problem section and a "Who's Who" of famous mathematicians of the past, not to men-

tion "Radius of the Lost Arc", the cartoon adventures of Polymath Jones.

*Quantum* contains English translations from *Kvant*, a Soviet student magazine published by the Academy of Sciences of the USSR and the Academy of Pedagogical Sciences, as well as new material in English; it is devoted to mathematics and physics in roughly equal proportions. Like *Mathematics Review*, the magazine is full of stimulating articles written in a style aimed at the 16–20 age group, although many would be as suitable for investigative reading for UK university courses as they would for A-level courses. The mathematics articles are generally less embedded in everyday life contexts and draw skilfully on the intrinsic mathematics to engage the interest of the reader. Again, the material is of good quality and well presented. Regular features include an analysis in recreational mathematics, an update on events and current activities of interest to science students, more particularly in the United States, and a selection of nice problems, ranging from the quick "Brainteasers" to the more meaty "Challenges" and "Math Investigations".

Together with *Mathematical Spectrum*, which sits somewhere between the two, *Mathematics Review* and *Quantum* provide excellent mathematical reading for students at school and university. In short, everything the teacher wanted to say about maths but was afraid to spend time on. □

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# From the horse's mouth

*Nigel Williams*

**Science and Public Affairs.** Editor Walter Bodmer. *Royal Society. 4/yr. UK £20, elsewhere £21.50, $43.*

THIS is not a new product but a relaunch last year in glossy-covered 'magazine' format of a previously small-format, scholarly looking journal. It is produced by the Royal Society in conjunction with the British Association for the Advancement of Science. The journal's original aims were to foster the understanding of scientific issues by the public and to explain the implications of discoveries in science and technology for everyday life. The journal is pursuing more of a mission than a gap in the market.

These rather grand aims broadly remain in the new format, which the editor, Walter Bodmer, hopes will make the journal (now, rather self-consciously, referred to as a magazine) "more inviting" and "easier to read for busy people". There is also more of it, with four issues promised each year.

The scope is necessarily broad, with engineering and technology (the actual 'science' that the public directly experiences) given welcome prominence alongside more basic topics such as cancer biology and cloud physics. Expertise abounds, but the quality of the writing is very uneven. Regular round-ups in areas of current interest are promised. "Embryos and ethics" has already appeared with a particularly lucid contribution from Sydney Brenner, but I think one would need to know some genetics to follow his argument fully.

The authors, mostly British, consist of a distinguished bunch of practitioners, administrators and industrialists with a scattering of lesser-knowns and journalists. If you want to know about British science policy issues, it is here that you are most likely to get a view from the horse's mouth. A new reviews section has yet to get properly under way.

Although the colourful cover could — just about — front something one could find at the newsstand, has the journal really transformed itself into a magazine? It is certainly more inviting with its attractive text layout and good modern typography, but some of the illustrations — dull publicity handouts and amateurish cartoons in particular — let it down.

Busy people will still read this 'magazine', but largely, I suspect, when they see a topic or author of interest, and most of the readers will be scientists. With many of the articles in excess of 3,000 words, it still has a low browse factor, which is a pity given the readership that some of the articles, such as Fran Balkwill's splendid account of the cytokines, deserve.

But scientists and policymakers can learn a little more than their own business. An account of the development of the compact disc at Philips reveals the enormous complexity of factors and timings involving in the currently fashionable area of innovation, and challenges glib links between science and technology.

The publication will be successful as a magazine, journal or whatever on the quality and clarity of what it says and who is saying it, but not on its format. □

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## Also submitted for review

The following is a list of journals received that were eligible for review but which for one reason or another are not covered in the preceding pages. The list does not include journals sent in that had not published enough titles to be considered.

*Antiviral Chemistry and Chemotherapy* (Blackwell Scientific)
*Applicable Algebra in Engineering, Communication and Computing* (Springer)
*Atomization and Sprays* (Hemisphere)
*Bio-medical Materials and Engineering* (Pergamon)
*Computational Mathematics and Modelling* (Consultants Bureau).
*Computing Systems in Engineering* (Pergamon)
*Designs, Codes and Cryptography* (Kluwer)
*Dreaming* (Human Science)
*Dynamics and Control* (Kluwer)
*European Journal of Information Systems* (Operational Research Society)
*Fish and Shellfish Immunology* (Harcourt Brace Jovanovich)
*Fresenius Environmental Bulletin* (Birkhäuser)
*Frontier Perspectives* (Center for Frontier Sciences at Temple University)
*Immunology and Infectious Diseases* (Rapid Communications of Oxford)
*International Journal of Algebra and Computation* (World Scientific)
*International Journal of Computational Geometry and Applications* (World Scientific)
*International Journal of Environmental Health Research* (Chapman and Hall)
*International Journal of Software and Knowledge Engineering* (World Scientific)
*Journal of Circuits, Systems, and Computers* (World Scientific)
*Journal of Global Optimization* (Kluwer)
*Journal of Information Systems* (Blackwell Scientific)
*Journal of Logic and Computation* (Oxford University Press)
*Journal of Micromechanics and Microengineering* (IOP)
*Journal of the Moscow Physical Society* (IOP)
*Journal of Systems Integration* (Kluwer)
*Mathematical Models and Methods in Applied Sciences* (World Scientific)
*Mechatronics: Mechanics – Electronics – Control* (Pergamon)
*Neuromuscular Disorders* (Pergamon)
*Optical Computing and Processing* (Taylor and Francis)
*Plasma Devices and Operations* (Gordon and Breach)
*Process Control and Quality* (Elsevier)
*Science, Technology and Development* (Frank Cass)
*Shock Waves* (Springer)
*Waves in Random Media* (Cambridge University Press)
*User Modeling and User-Adapted Interaction* (Kluwer)

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**MUNICH** – Matthew Beard, Sandstrasse 41 8000 München 2. Telephone: (089) 52 40 82. Fax: (089) 5 23 22 22.
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## TENURED AND TENURE-TRACK ASTRONOMERS

The Space Telescope Science Institute (STScI) has several openings for tenure-track astronomers. Excellence in research is the primary qualification for these positions. The level of the appointments – assistant, associate, or full astronomer – will be determined by the experience and accomplishments of the successful applicants. Tenure-track astronomers at STScI spend about 50% of their time in personal research and the remainder in support of the science mission of the Hubble Space Telescope. There is some latitude within this framework to tailor the functional component of the positions to the interests and expertise of the successful applicants.

STScI supports the research of its scientific staff through visitor, postdoctoral, and graduate-student programs. Several workshops and symposia are held each year at the Institute. Internal funds are available for computing and other equipment. Members of the staff may obtain additional support from any of the regular NASA grant programs. The salaries and benefits of tenure-track astronomers are commensurate with those at AURA member universities (without the need to apply for summer support).

Candidates for these positions must have a Ph.D. or equivalent degree in Astronomy, Physics, or Planetary Science and some postdoctoral experience. STScI is committed to equal opportunity; women and minorities are strongly urged to apply. Candidates should send a curriculum vitae and bibliography, a brief statement of research and functional interests, and the names of four references to the **Personnel Manager, Space Telescope Science Institute, 3700 San Martin Drive, Baltimore, Maryland 21218. Applications received by 15 December 1992 will receive full consideration.**

SPACE TELESCOPE SCIENCE INSTITUTE

(NW8455)A

NYU Medical Center

## Assistant Research Scientist

NYU Medical Center currently has a post-doctoral position available in our Lipid Metabolism Laboratory. The successful candidate will conduct research in human vitamin E metabolism using deuterated tocopherols. Regulation of plasma, lipoprotein and tissue concentrations, as well as discrimination and function of the hepatic tocopherol binding protein. Training in biochemistry/molecular biology with emphasis on lipoprotein metabolism is desirable.

Please send your resume to Maha Singh, Human Resources Dept., NYU Medical Center, 550 First Ave., New York, NY 10016. EOE, MF.

(NW8467)A

## COMMUNITY ECOLOGIST

The Department of Zoology, University of Florida, announces a position at the assistant professor level for an ecologist with research interests in tropical communities. Duties will include teaching introductory biology and general ecology, developing a graduate course in community ecology, supervising graduate student research, and interacting with faculty in the Zoology Department and in other UF academic units that have ecological or tropical orientations. Each applicant should submit a curriculum vitae; names, addresses and telephone numbers of four references; a synopsis of research interests; and a detailed outline of an undergraduate ecology course. Send application materials by November 30, 1992 to **Dr. Brian K. McNab, Chair, Community Ecology Search Committee; Department of Zoology; University of Florida; Gainsville, Florida 32611.**

*Filling this position is contingent upon the availability of state funds. The University of Florida is an equal opportunity/affirmative action employer.*

(NW8457)A

## THE UNIVERSITY OF MANCHESTER
## CHAIR OF DERMATOLOGY

The University invites applications for a full-time Chair of Dermatology from candidates with medical qualifications allowing clinical practice in the United Kingdom. Salford Health Authority is prepared to offer an Honorary Consultant contract to a suitably qualified, successful applicant.

The Chair is a new appointment, presenting an exceptional opportunity for a dynamic individual who is a major contributor to research in Dermatology and able to provide academic leadership in this important specialty.

**Applications (one copy suitable for photographic reproduction), giving full details of qualifications and experience, together with the names and addresses of three persons to whom reference may be made, should be sent, not later than November 30th, 1992, to the Registrar, The University, Manchester, M13 9PL, from whom further particulars may be obtained (tel: 061-275 2028). Quote Ref 181/92. Overseas candidates may apply by facsimile (no. 061-273 5306) in the first instance. The University is an equal opportunity employer.**

(9410)A

# STEM CELL BIOLOGY

Amgen has distinguished itself as the industry leader in the development of human therapeutic products through applications of biotechnology. As a result of our continuing commitment to the discovery and characterization of novel hematopoietic growth factors, we are actively seeking Research Scientists and Research Associates to contribute to the development of this program.

## Research Scientists

Candidates for the Research Scientist positions must have a Ph.D. in cell/molecular biology or biochemistry, preferably with an emphasis on hematopoietic cell growth and differentiation, and two to four years post-doctoral experience. Responsibilities for this position will include development of new research programs, in particular, the identification and characterization of new hematopoietic growth factors, stem cell and megakaryocyte biology and receptor biology, as well as complementing on-going projects. Please respond to **Job Code #N-402**

## Research Associates

Two Research Associate positions are available in the department of stem cell biology. These positions will require the candidates to be part of a research team whose goal is to increase our understanding of hematopoietic stem cell biology and develop assay/culture systems which will lead to the identification of novel cytokines.

Candidates for this position must have a B.S./M.S. in biochemistry, cell biology or a related field and at least two years of research experience, preferably with emphasis on hematopoiesis and/or immunology. Experience in protein characterization, assay development, and work with animals is desirable. Please respond to **Job Code #N-503**

Candidates for this position must have a B.S. in biology, biochemistry, microbiology, physiology or a related field. At least one year of research experience is required which must include cell culture and animal handling ability. Please respond to **Job Code #N-504**

**Amgen provides a stimulating and challenging research environment** in which individuals are given the resources necessary to achieve their goals. Our compensation and benefits package reflects our commitment to attracting the best scientific talent available. If you have the necessary skills and would like to be a part of an organization that places a high priority on its human resources, please send a complete CV, in confidence, to: **Amgen Inc., Staffing Department (Mail Code 10-1-A-411), (indicating the job code number), Amgen Center, Thousand Oaks, CA 91320-1789.** Amgen is an equal opportunity employer.

AMGEN®

Principals only, please.

(NW8480)A

# MOLECULAR BIOLOGISTS

Merck Research Laboratories is seeking several molecular biologists to join our Department of Genetics and Molecular Biology. Successful candidates will join a group of outstanding scientists developing programs on mechanism-based drug research.

Requirements include having expertise in nucleic acid manipulation, tissue culture and cell biology. You should also have a B.S./M.S. or equivalent in Genetics, Microbiology or Biochemistry.

Salary, benefits and growth potential for these positions are excellent. The work location is in Rahway, New Jersey, approximately 25 miles from New York City. Please address your resume and the names of three references to **Dr. L.H.T. Van der Ploeg, Director, Department of Genetics and Molecular Biology, # N47, c/o Human Resources, MERCK RESEARCH LABORATORIES, P.O. Box 2000, WBC-155, Rahway, NJ 07065.** An EEO/AA/VH Employer.

(NW8484)A

MERCK
Research Laboratories

# ASSISTANT MEMBER

## Memorial Sloan-Kettering Cancer Center

The Program in Molecular Biology, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center invites applications for two positions as Assistant Member. The successful candidates will have demonstrated outstanding potential for a research career and will be using a combination of molecular genetic, cell biological, or modern biochemical approaches to study either 1. the cell cycle or 2. developmental genetics of vertebrates.

Applications should include a curriculum vitae, a summary of current research activities and future research plans, and three letters of references. Please send applications concerning vertebrate developmental genetics to **Dr. Elizabeth Lacy or Dr. Peter Besmer, Co-Chairs, Developmental Genetics Search Committee, Program in Molecular Biology, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021.** Applications concerning the cell cycle should be directed to **Dr. Kenneth J. Marians, Chairman, Molecular Biology, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021.** All applications must be completed by **December 1, 1992.**

*SKI/MSKCC is an Equal Opportunity Employer.*

(NW8335)A

# 山之内製薬 研究員募集

国際的な研究ネットワークの構築、基礎研究力の強化など、独創的な新薬の創造に向けて、山之内製薬の研究環境はますます充実。21世紀をともに担う意欲的な研究員を募集します。

**中央研究所　（筑波）**

**専門分野：分子生物学、薬理学**

分子薬理学的あるいは蛋白工学的研究手法を基盤に、学際的な創薬研究に積極的に参加したい方を募集します。

年　齢：27～35才

資　格：Ph.Dまたは同等の能力・経験のある方

Post doc.の経験があればさらに好適です。

**リサーチセンター（東京）**

業務内容：医薬品の研究開発の企画、情報収集、評価及び推進

年　齢：27～32才

資　格：Ph.Dまたは同等の能力・経験のある方

待遇は本社規定により優遇、入社時期等詳細は応相談。応募者は履歴書、研究業績目録、主要論文別刷を下記宛にご郵送下さい。秘密は厳守します。

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山之内製薬

(W0087)A

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UNSW
THE UNIVERSITY OF NEW SOUTH WALES
SYDNEY, AUSTRALIA

*Equality of employment opportunity is University policy*

**SCHOOL OF ANATOMY**

## ASSOCIATE LECTURER/LECTURER

**Ref. 392 — Tenured or Tenurable Appointment**
**Ref. 393 — Fixed Term Appointment**

**Salary: Associate Lecturer A$28,700 — A$38,950 per annum;**
**Lecturer A$41,000 — A$48,688 per annum.**

The School of Anatomy has vacancies for two academic staff. Appointment will be made either at the level of associate lecturer or lecturer. Level of appointment and commencement salary are dependent on qualifications and experience. A clinical loading of A$13,484 per annum will also be payable to an appointee at Lecturer level with medical qualifications registrable in NSW. The School is responsible for teaching gross anatomy, histology, neuroanatomy and embryology to medical and science students. It has an active research program with particular strengths in neuroanatomy and developmental neurobiology.

The appointees will be required to teach gross anatomy and at least one other subject, and to develop a research program with the potential to attract external funding.

The minimum qualification for Associate Lecturer is a good Honours degree in a relevant discipline, or a medical degree. A Lecturer should, in addition, have a relevant PhD or equivalent. Experience in teaching, research or clinical practice would be an advantage.

**Ref. 392** — Tenured or Tenurable: Appointment will either be with tenure or on the basis of contract with provision for conversion to tenure.

**Ref. 393** — Fixed Term: Appointment is on the basis of a fixed term of 3 years.

*Applicants must indicate the level and type of appointment for which they wish to be considered by quoting the relevant reference number.*

The positions are available from 1 February 1993. Membership of a University approved superannuation scheme is compulsory for new employees.

Further information from Associate Professor David Tracey on telephone (61 2) 697 2471.

Applications close 6 November 1992.

PLEASE QUOTE Appropriate Ref 392XX/393XX

### APPLICATION PROCEDURE

***Applicants should submit a written application QUOTING REFERENCE NUMBER. Include business and private telephone numbers; a complete resume, (copies of academic transcript and qualifications where appropriate); and the names, addresses (and preferably facsimile numbers) of at least two referees to: The Recruitment Officer, Staff Office, P.O. Box 1, Kensington, N.S.W. 2033 by applications close date. People from EEO groups are encouraged to apply.***

(W0158)A

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**UNIVERSITY OF ABERDEEN**

## RESEARCH ASSISTANT/ FELLOW

***£12,129-£19,328***
***(Pay award pending)***

required for the Department of Chemistry to work with Professor F P Glasser on the development of dense, chemically-resistant modified portland cement. This project, based in the Department of Chemistry is collaborative with Danish and German partners under the auspices of the BRITE-EURAM programme will commence in early 1993.

You should hold a PhD degree and/or have relevant experience in the above field.

The post is tenable for 27 months.

For further details and informal discussion telephone 0224 272906.

**Application forms and further particulars are available from: Personnel Services, University of Aberdeen, Regent Walk, Aberdeen AB9 1FX, telephone 0224 272727 quoting reference number ZCM 024R. A 24 hour answering service is in operation.**

*Closing date: 6th November, 1992.*

An equal opportunities employer.

(9560)A

## Department of Genetics

## Case Western Reserve University School of Medicine

The Department of Genetics invites applications for up to four tenure-track positions at the Assistant Professor, Associate Professor, or Professor rank as part of a major expansion. Applicants should have demonstrated expertise and productivity in any area of classical or molecular genetics, with ability to develop a strong, independent research program. Existing laboratories in the department focus on the genetics of Drosphila, C. elegans, mouse, and humans. Areas of concentration in the department will be in developmental genetics, chromosome structure and function, genome organization and mapping, and the genetics of inherited disease. Planned expansion is supported by significant new resources, including 40,000 square feet of state-of-the-art research space in a new Biomedical Research Building scheduled to open in January 1993, with generous equipment and start-up funds provided by the Markey Center for Developmental Genetics and by the Center for Human Genetics. The Department of Genetics participates in the multidepartmental Biomedical Sciences Training Program for Ph.D. students. Candidates will be expected to participate in the teaching of medical and graduate students. Strong interdepartmental interactions at Case Western Reserve University School of Medicine are encouraged and provide for an unusually supportive research environment and opportunity for achievement.

Applicants should send curriculum vitae, description of research plans, and names of three references (with contact numbers) to: **Dr Huntington F Willard, Chairman, Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH 44106-4955.**

*Case Western Reserve University is an Equal Opportunity/Affirmative Action employer.*

(NW8466)A

## FACULTY POSITIONS

### IN CELL, MOLECULAR AND STRUCTURAL BIOLOGY

Applications are invited for tenure-track positions in the Department of Cell, Molecular and Structural Biology at Northwestern University Medical School. **ASSISTANT TO FULL PROFESSOR** applicants must have a Ph.D., M.D., or equivalent degree, with research experience in cell, molecular, or structural biology. Applicants will be screened with respect to their ability to carry on vigorous independent research and to participate in the various teaching programs administered by the department. Candidates whose research interests include developmental biology, biochemistry, and structural biology are encouraged to apply, although outstanding applicants with research interests in other areas of molecular and cell biology will be considered. Send complete curriculum vitae and a brief description of research interests to: **Dr. Rex Chisholm, Chairman, Search Committee, Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611.**

*Northwestern University is an Equal Opportunity/Affirmative Action Educator and Employer and invites applications from all qualified individuals. Applications from women and minorities are especially sought.*

(NW8472)A

香 港 大 學

## THE UNIVERSITY OF HONG KONG

## CHAIR OF PHYSICS

*(Ref: 91/92-86)*

Applications are invited for appointment to the Chair of Physics, vacant from September 1, 1992 on the departure of Professor W Y Chau. Applicants should have strong academic qualifications and a substantial record of research and publications. Appointees to Chairs may be called upon to become Head of Department.

The University would prefer to make a permanent appointment, but consideration may also be given to applications for appointment for a fixed-term or on secondment for a period of preferably not less than three academic years. The University reserves the right not to fill the Chair or to fill the Chair by invitation or to make an appointment at a lower level.

Annual salary (superannuable) will be within the professorial range, of which the minimum is HK$762,240 and the average is HK$942,780 (approx £52,750 & £65,244 respectively; sterling equivalent as at 14 September, 1992). At current rates, salaries tax will not exceed 15% of gross income. Housing at a charge of 7.5% of salary, children's education allowances, leave and medical benefits are provided.

**Further particulars and application forms may be obtained from Appointments (41053), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK, or from the Appointments Unit, Registry, The University of Hong Kong, Hong Kong (Fax (852) 559 2058; E-mail: APPTUNIT@HKUVM1.-HKU.HK).**

*Closes: 31 December 1992.*

(W0164)A

## IFIAS

## Executive Director

The International Federation of Institutes for Advanced Study (IFIAS) invites applications for the post of Executive Director to succeed Professor Ian Burton. The position is initially available for up to three years at the IFIAS Secretariat in Toronto. Candidates who wish to apply on a second basis will also be considered.

IFIAS is an association of 44 diverse member organizations from 27 countries including research institutes, corporations and public agencies, carrying out activities that address world problems by linking science to policy, in a creative, non-routine mode. The Executive Director is responsible to an international board for the overall direction of IFIAS including strategic planning, fundraising, financial management, research programmes, and international seminars and public symposia. The Director also maintains the flow of information between members, represents IFIAS internationally, and negotiates grants and commitments with donor organizations including government agencies. The Director is assisted by a Deputy Director and programme development officers in Toronto, and by member organizations. Salary according to qualifications and experience.

Send curriculum vitae and names of three references to:

**Chair, Director Selection Committee,**
**IFIAS,**
**39 Spadina Road,**
**Toronto, Ontario, Canada M5R 2S9**

by October 30th, 1992. Selected candidate will be asked to take up the position on January 1, 1993, or as soon as possible thereafter.

(NW8464)A

# UNIVERSITY OF KUWAIT
# HEALTH SCIENCE CENTRE

## FACULTY OF ALLIED HEALTH SCIENCES & NURSING

## Teaching Appointments in Medical Laboratory Technology

Kuwait University seeks motivated individuals who are attracted by the challenge of preparing young professionals to lead one of the world's fastest developing health care systems into the 21st century.

The Faculty of Allied Health Sciences and Nursing offers 4-year University-based B.Sc. programmes, with practical training carried out in local hospitals. Appointees to all posts will participate in both theoretical and practical teaching, and development and management of their respective programmes. The language of instruction is English.

### Positions

a) **Chairman:** At the level of Professor with expertise in any of the disciplines taught within the department and strong administrative experience required.

b) **Haematologist:** At the level of Assistant or Associate Professor — clinical laboratory experienced required.

c) **Clinical Chemist:** At the level of Assistant or Associate Professor.

d) **Histologist:** At the level of Assistant or Associate Professor — experience in both microscopic anatomy and histotechnology required.

e) **Microbiologist:** At the level of Assistant or Associate Professor.

f) There are also a limited number of vacancies for Lecturers and Senior Lecturers.

### Requirements for appointment

1. **Professors, Associate Professors and Assistant Professors**
   All applicants must possess a Ph.D., or equivalent qualification, in their speciality. Professors must have 14 years' experience since their B.Sc., including 8 years since their Ph.D., and have served at least 4 years as an Associate Professor. Associate Professors must have at least 9 years' experience since their B.Sc., including 4 years since their Ph.D., and served at least 4 years as an Assistant Professor. Candidates for Professor and Associate Professor should have a substantial record of research publications in journals of international repute.

### Salary

1. Total monthly salary within the following scales:

| | | |
|---|---|---|
| Professor | KD.1070 - 1230 | ( 8 annual increments) |
| Associate Professor | KD. 875 - 1035 | ( 8 annual increments) |
| Assistant Professor | KD. 680 - 840 | ( 8 annual increments) |
| Senior Lecturer | KD. 670 - 820 | (10 annual increments) |
| Lecturer | KD. 545 - 695 | (10 annual increments) |

**1KD. = US$3.45; UK 2**

There is no income tax in Kuwait, and currency is transferable without restriction.

### Other Benefits

1. In addition, for teaching staff who have an active part in the Ministry of Public Health programme, there is a monthly supplement (Lecturer, KD.100; Senior Lecturer, KD.125; Assistant Professor, KD.220; Associate Professor, KD.285; Professor, KD.350) for 10 months a year paid by the Ministry.
2. An attractive package of associated benefits includes free furnished accommodation, 60 days paid summer leave and 10 days mid-year break for teaching staff, round-trip air ticket, end-of-service gratuity, free medical care in Kuwait government hospitals.
3. Professors, Associate Professors and Assistant Professors also receive a generous baggage and freight allowance, and education allowances for up to 3 children, and may attend one approved conference per year. A social allowance (KD.65-87 after deduction) is also payable.

### Applications

Applications in duplicate, including full curriculum vitae, personal details, comprehensive publication list, 2 recent passport photographs and the names and addresses of 3 referees, should be sent to:

**The Dean**
**Faculty of Allied Health Sciences & Nursing**
**Kuwait University Health Science Centre**
**P.O. Box 31470**
**90805 Sulaibikhat**
**Kuwait.**

*Closing date for applications: 15 December, 1992. Late applicatio*

(W0157)A

**UNIVERSITY OF LEICESTER**
**Centre for Mechanisms of Human Toxicity**
**Biological NMR Centre**
**UNIVERSITY OF DUNDEE**
**Biomedical Research Centre**

## Post-Doctoral Research Associates *and* Research Technician Structural and Functional Analysis of Drug-metabolizing Enzymes

Applications are invited for three research positions to work in a programme aimed at elucidating the molecular basis of the specificity of drug-metabolizing enzymes, including cytochrome $P_{450}$ and glutathione S-transferase, using a combination of structural methods (principally NMR spectroscopy), molecular biology and protein biochemistry. These positions are funded by a Medical Research Council programme grant and are available for up to five years.

1. **Post-doctoral molecular biologist (Dundee).** To work in the laboratory of Professor Roland Wolf on the cloning, high level expression and mutagenesis of genes coding for drug-metabolizing enzymes, particularly cytochrome $P_{450}$. Experience of recombinant DNA techniques will be an advantage.
2. **Post-doctoral NMR spectroscopist/protein biochemist (Leicester).** To work in the laboratory of Professor Gordon Roberts on the structural and biochemical aspects of the programme. Applications are invited *either* from NMR spectroscopists with experience of working with proteins *or* from experienced protein biochemists with an interest in NMR spectroscopy.
3. **Research Technician grade C (Leicester).** To work primarily on the purification and characterization of recombinant enzymes, including enzymes labelled with stable isotopes; some molecular biological work will also be involved. Applicants should possess an ONC, BTEC or equivalent and have at least three years' relevant experience; recent graduates will also be considered for appointment as Graduate Trainees. Experience of protein purification is essential.

Salaries for positions 1 and 2 will be on the R&A IA scale, with a starting salary in the range £12,129-£19,328 pa (under review). For post 3, the starting salary will be in the range £9,488-£10,639 pa according to experience and qualifications; graduate trainees will receive an initial £8,588 pa. All three posts are available with effect from 1 October 1992.

**Applications, including CV, list of publications and the names and addresses of two referees, should be sent, *for post* 1 to Professor CR Wolf, Biomedical Research Centre, University of Dundee, Ninewells Hospital, Dundee, Scotland, and *for posts* 2 *and* 3, to the Secretary, Biological NMR Centre, University of Leicester, PO Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN, UK. Informal enquiries to Professor Wolf (031-668-3343) or Professor Roberts (0533 522938) are welcome. Closing date for receipt of applications: 22 October 1992.**

*Towards Equal Opportunities.* (9537)A

## TENURE TRACK ASSISTANT PROFESSOR OF BIOCHEMISTRY

**UNIVERSITY OF WASHINGTON**

Demonstrated ability to develop a strong and creative research program in any area of modern biochemistry, including but not limited to biophysical and molecular approaches to biological regulatory processes and macromolecular function. Ph.D. degree or its equivalent and appropriate postdoctoral research experience will be considered. Send CV, copies of representative papers, a succinct statement of research interests, and three letters of recommendation by November 30, 1992 to:

**Search Committee, Department of Biochemistry, SJ-70, University of Washington, Seattle, WA 98195; FAX: 206-685-1792.**

*The University of Washington is an Affirmative Action/Equal Opportunity Employer.*

## CYTOGENETICIST

The Department of Pediatrics and Human Development Michigan State University has an opening for a Cytogeneticist. This person's primary responsibility will be to direct the clinical Cytogenetics Laboratory. This laboratory currently karyotypes about 2,400 specimens a year including amniocentesis, blood, tissue and marrow samples. The Cytogenetics laboratory is part of the Clinical Genetics Unit and the laboratory Director will interact closely with other faculty and staff in that unit. There will also be significant interaction with other individuals in this Department and with other Departments on campus. The successful candidate will be expected to develop and introduce new cytogenetic technology as it becomes available. They will be expected to pursue scholarly activity either in their own research program or in collaboration with others. It is also anticipated that they will spend a small amount of time teaching medical students and residents. A full time, 12 month academic appointment is jointly offered with the Department of Pathology. Academic position and salary according to experience.

Requirements: M.D. and/or Ph.D. in an appropriate Biological Science. Certification in Cytogenetics from AAHG.

For further information please contact: **Dr. Rachel Fisher Search Committee Chair, Dept.Ped./Hum. Dev Michigan State University, B245B Life Sciences, East Lansing MI 48824. (517) 353-2008.**

*Michigan State University is an Affirmative Action/Equal Opportunity Institution.* (NW8461)A

## ESA ASTRONOMERS

The Space Telescope Science Institute (STScI) expects to have openings for astronomers. Excellence in research is the primary qualification for these positions. The level of the appointments will be determined by the experience and accomplishments of the successful applicants. ESA astronomers at STScI spend about 50% of their time in personal research and the remainder in support of the science mission of the Hubble Space Telescope. There is some latitude within this framework to tailor the functional component of the positions to the interests and expertise of the successful applicants.

STScI supports the research of its scientific staff through visitor, postdoctoral, and graduate-student programs. Several workshops and symposia are held each year at the Institute. Internal funds are available for computing and other equipment.

Candidates for these positions must be nationals of ESA member states and must have a Ph.D. or equivalent degree in Astronomy, Physics, or Planetary Science and some postdoctoral experience. Candidates should send a curriculum vitae and bibliography, a brief statement of research and functional interests, and the names of four references to Dr. D. Macchetto, European Space Agency, Space Telescope Science Institute, 3700 San Martin Drive, Baltimore, Maryland 21218. Applications will be accepted until 15 December 1992. EEO/AA M/F/D/V

SPACE TELESCOPE SCIENCE INSTITUTE

(NW8458)A


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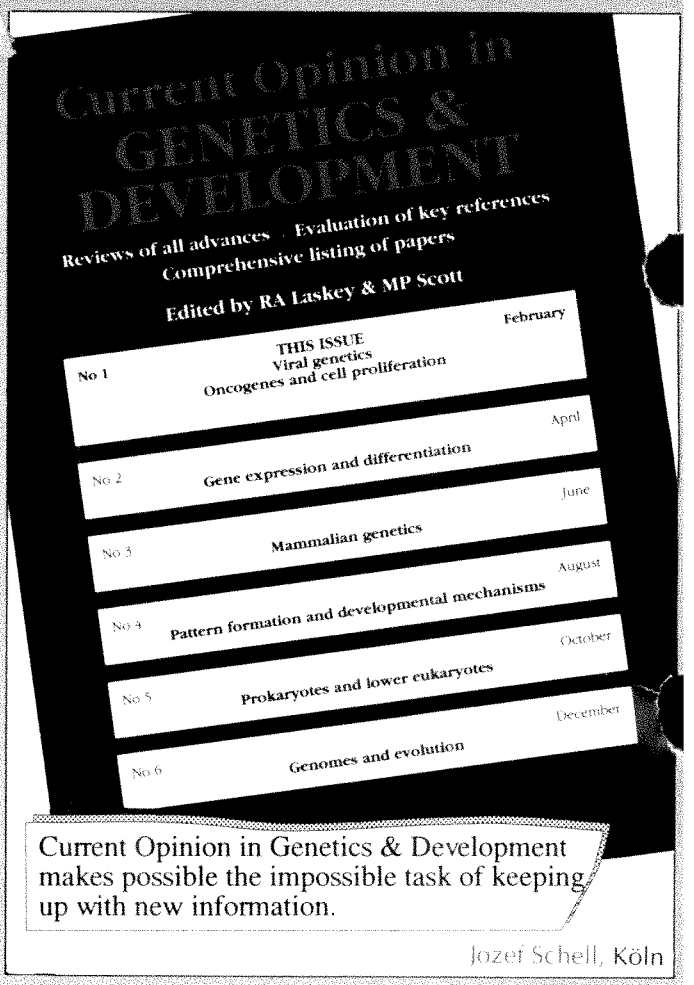


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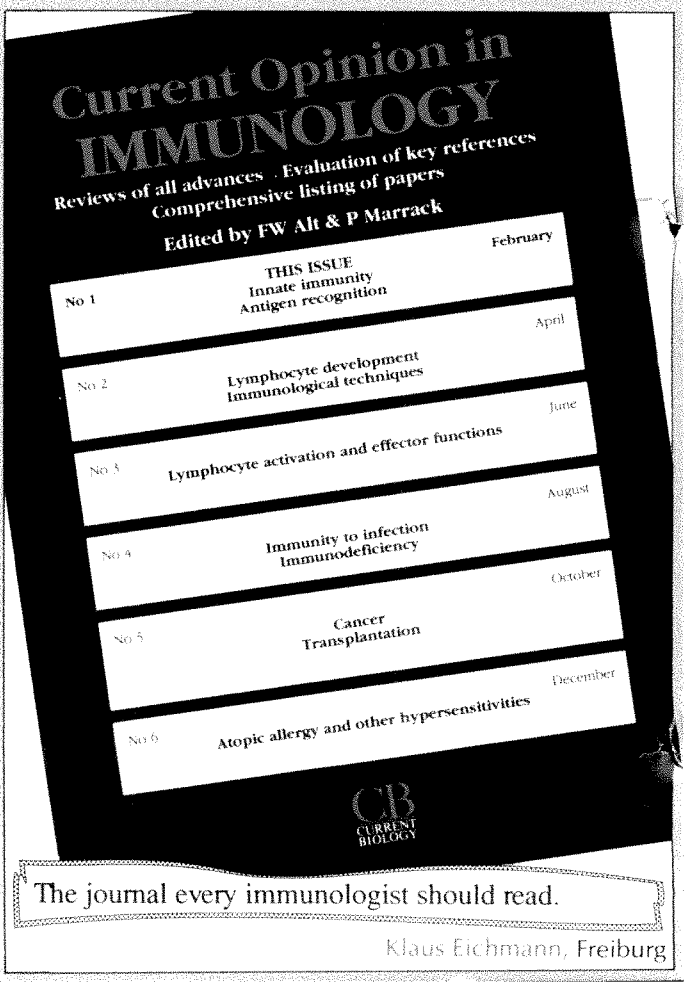


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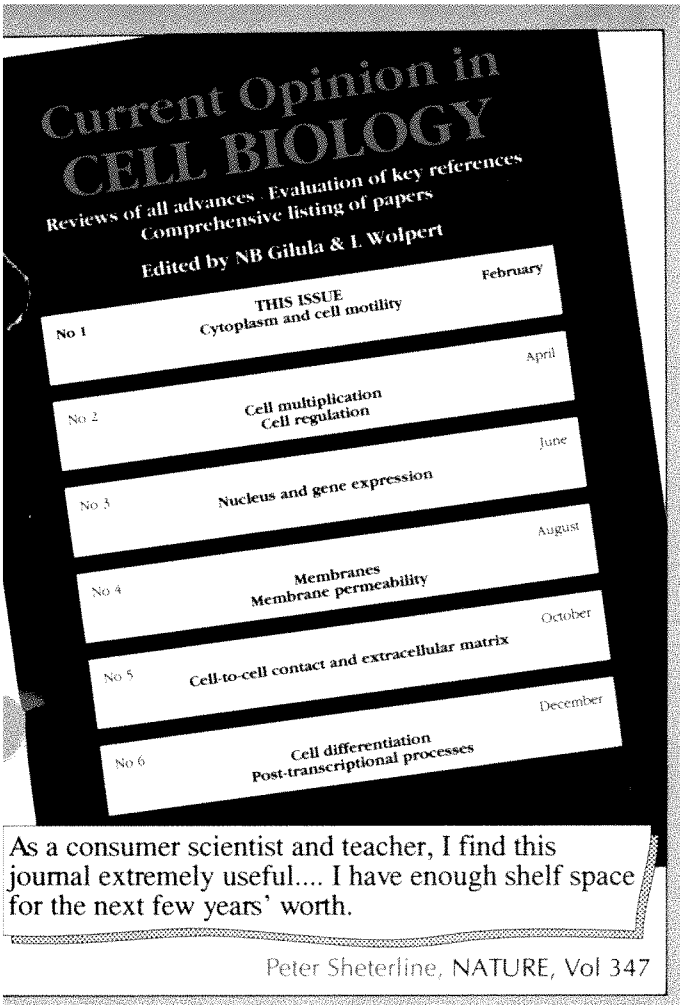
Royal Melbourne Hospital,
Melbourne

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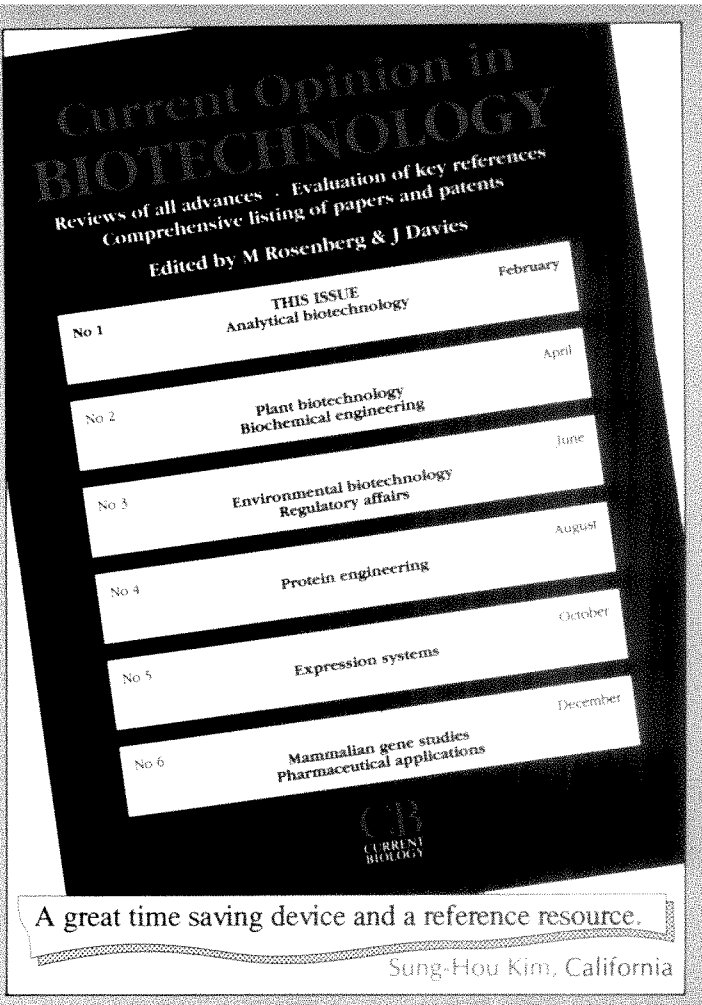
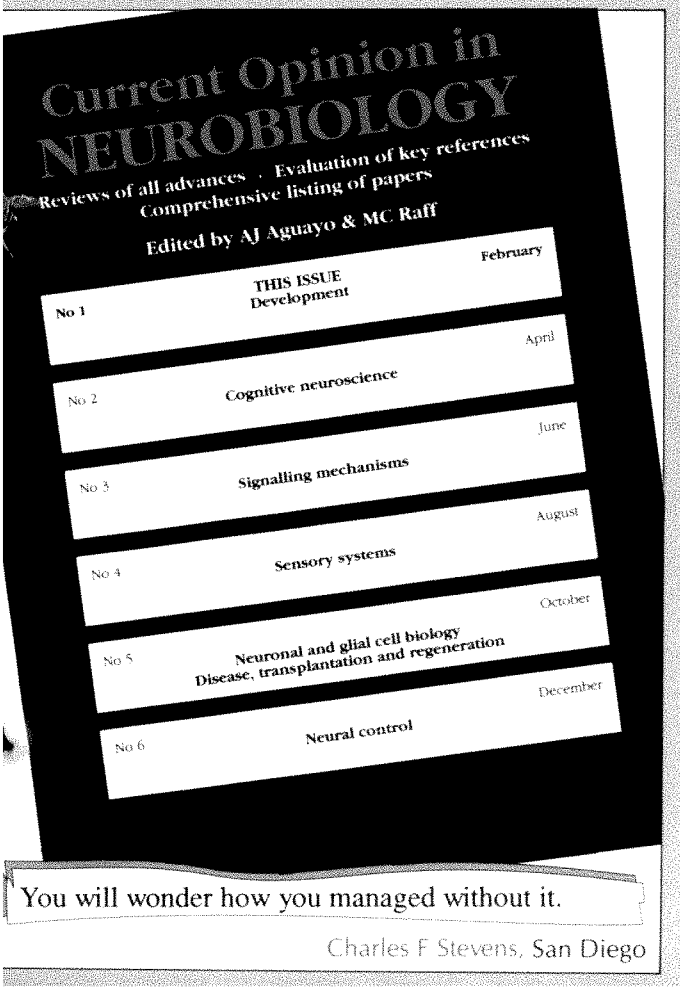
Peter Sheterline, NATURE, Vol 347



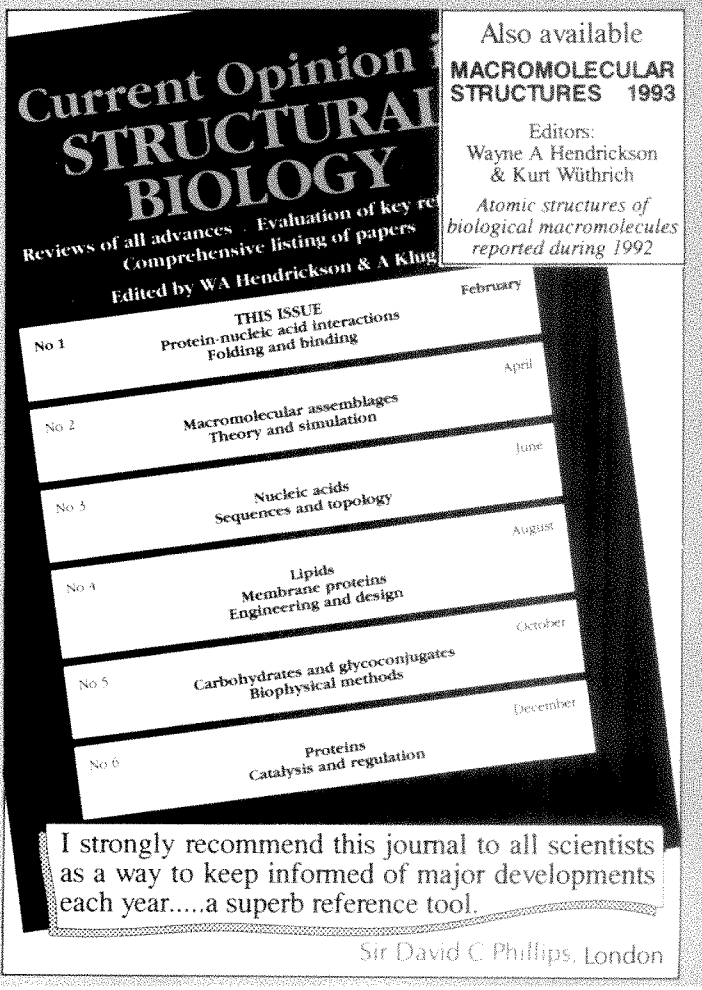

A great time saving device and a reference resource.

Sung-Hou Kim, California




You will wonder how you managed without it.

Charles F Stevens, San Diego

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**MACROMOLECULAR STRUCTURES 1993**

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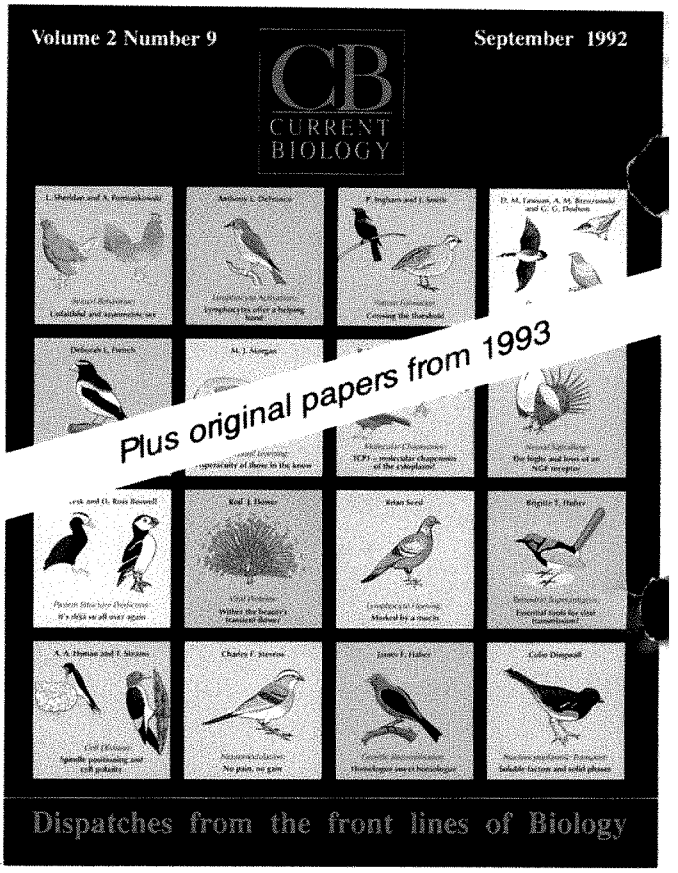




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# Research Scientist - Population Modelling

$A41,929 - $A56,531 + Superannuation

*Division of Fisheries, Hobart, Tasmania, Australia*

We are seeking an experienced and innovative scientist to research the population dynamics of commercially important temperate shark stocks in Australian waters.

Studies are currently directed towards an investigation of the spatial structure of the fishery including quantification of movement and mixing rates from tagging experiments, the evaluation and development of a catch/effort database, refining models of catchability, and evaluation and risk analysis of management strategies. The appointee will play a major role in a multi-organisational research program on southern shark and in scientific and management discussions relevant to the fishery.

You will need a PhD or equivalent experience in quantitative zoology, fisheries biology, or similar, together with a strong background in the development of mathematical and computer models for biological systems.

You should have a superior record of innovative research achievements and the capacity to communicate research findings to a wide variety of audiences.

Dr John Stevens telephone (international) 61-02-206353 can provide further information and our selection criteria. The position is indefinite (tenured) and assistance with relocation costs is provided.

Please send applications addressing the selection criteria and including details of your qualifications and experience and the names of two referees, quoting Ref. No. FC92/25 NAT to: **The Human Resources Manager, CSIRO Division of Fisheries, GPO Box 1538, Hobart, Tasmania 7001, Australia** by November 20, 1992. (W0144)A

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## POSTDOCTORAL OR RESEARCH ASSOCIATE

Position to investigate the regulation of the nitric oxide-guanylyl cyclase signalling pathway in pulmonary vascular physiology and pathophysiology. Molecular biology expertise required. Send C.V. to **Dr. Roger A. Johns, Box 238, University of Virginia Health Sciences Center, Charlottesville, VA 22908 USA.** *An Equal Opportunity/Affirmative Action Employer.* (NW8469)A

## University of Reading
## School of Animal & Microbial Sciences

Postdoctoral molecular biologist required for 2 years to work with Prof. K. Simkiss studying germ cell proliferation in birds. *Apply for application form* to Personnel Officer, University of Reading, Whiteknights, P.O. Box 217, Reading RG6 2AH. Tel: (0734) 318754. Please quote ref. R9241. (9539)A

# University of Nottingham

## Department of Genetics

# Professor and Head of Department

The University is seeking an outstanding scholar to head the Department in succession to Professor Bryan Clarke, FRS, who has been appointed to a Research Chair.

Both the University and the Department are unequivocally committed to the support of basic research at a high level. They are interested in enhancing the already high standing of the Department recognised in the last Research Assessment Exercise and in the support that it receives from the Research Councils.

The Department is exceptionally well equipped for research in molecular and population genetics.

An additional Lectureship will be advertised after consulation with the new Professor.

Informal enquiries to Professor B C Clarke FRS or Professor R G Lloyd, Department of Genetics, Queen's Medical Centre, Nottingham NG7 2UH (tel 0602 420639 (Clarke) or 0602 709406 (Lloyd); FAX 0602 422225).

Further details and application forms, returnable not later than 31 October, from the Personnel Officer, University of Nottingham, University Park, Nottingham NG7 2RD (tel 0602 515780 or FAX 0602 515205). **Ref No 1568.** (9532)A

# Bioscience and Neutrons

Applications are invited for the position of Research Associate at Chalk River Laboratories in the physics of soft condensed matter (biological systems, emulsions, rubbers, liquid crystals). The successful candidate will join the existing neutron scattering group at Chalk River and will collaborate in developing the bioscience program. The neutron scattering group utilizes triple-axis spectrometers, a powder diffractometer and a small angle scattering spectrometer at the NRU reactor. It has extensive collaborations with university and industrial scientists including research on lipid bilayers. Candidates with a PhD in any related field will be considered. Experience with neutron scattering methods would be an advantage but is not essential. This advertisement is directed primarily to Canadian citizens or permanent residents, but all qualified candidates are encouraged to apply. Please forward a curriculum vitae and publication list, with two letters of reference, by October 30, 1992, quoting File No. CSP2119, to:

Dr. M. Harvey
Director, Physics Division
AECL Research
Chalk River Laboratories
Chalk River, Ontario
Canada K0J 1J0

(NW8479)A

## POST-DOCTORAL POSITIONS

available immediately for molecular studies of transcription factor expression and function in hematopoiesis. Research will include characterizing recently cloned transcription factors and identifying additional transcription factors important in lymphokine gene expression and leukemogenesis. Applicants should have a PhD degree and background in gene expression, DNA-protein interactions, signal transduction, orprotein purification.

**Send a letter of interest, CV and names and phone numbers of two references to: Dr Stephen D Nimer, c/o Memorial Sloan-Kettering Cancer Center, Department of Medicine, 1275 York Ave, New York, NY 10021.**

*Memorial Sloan-Kettering Cancer Center is an equal opportunity employer.* (NW8485)A

# ERATO

Exploratory Research for Advanced Technology

**Research Development Corporation of Japan**

## BASIC RESEARCH

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The Research Development Corporation of Japan, a statutory corporation of the Japanese government, is receiving applications for the ERATO projects listed below from young scientists interested in recognized, well-funded, multidisciplinary research. ERATO projects operate for five-year terms and are independent from the Project Directors' home institutions. Researchers come from universities, government laboratories, industry, and overseas.

### Kawachi MILLIBIOFLIGHT Project

Researchers in the fields of **DYNAMICS, FLUID DYNAMICS, STRUCTURAL MECHANICS, CONTROL, BIOLOGY, PHYSIOLOGY, and PRECISION MECHANICAL ENGINEERING** are needed to research the flying and swimming of organisms at low Reynolds numbers. Project Director: Prof. Keiji Kawachi, The University of Tokyo.

### Itaya ELECTROCHEMISCOPY Project

Researchers in the fields of **ELECTROCHEMISTRY, SPECTROELECTRO-CHEMISTRY, SURFACE SCIENCE, HIGH-VACUUM DEPOSITION, and SEMICONDUCTOR PHYSICS** are needed to use *in-situ* STM and AFM to research metal and semiconductor surfaces in aqueous and nonaqueous solutions. Project Director: Prof. Kingo Itaya, Tohoku University.

### Yanagida BIOMOTRON Project

Researchers in the fields of **BIOPHYSICS, PROTEIN ENGINEERING, GENETIC ENGINEERING, and INSTRUMENTATION (NANOMETER MEASUREMENTS, LASER OPTICS, PROBES, HIGH-SPEED IMAGING, LOW-LEVEL LIGHT DETECTION)** are needed to research the mechanisms and energetics of the actin-myosin complex. Project Director: Prof. Toshio Yanagida, Osaka University.

### Yoshizato MORPHOMATRIX Project

Researchers in the fields of **DEVELOPMENTAL BIOLOGY, CELL BIOLOGY, MOLECULAR BIOLOGY, and BIOCHEMISTRY** are needed to research the role of the Extracellular Matrix (ECM) in regeneration and metamorphosis in animals. Project Director: Prof. Katsutoshi Yoshizato, Hiroshima University.

### Fusetani BIOFOULING Project

Researchers in the fields of **NATURAL PRODUCTS CHEMISTRY, MARINE BIOLOGY, ELECTROPHYSIOLOGY, and BIOCHEMISTRY** are needed to research the signal chemistry and electrophysiology of sessile organisms such as coral and mussels. Project Director: Prof. Nobuhiro Fusetani, The University of Tokyo.

### Information Qualifications Applications

The **ERATO GUIDE FOR PROSPECTIVE RESEARCHERS — 1992** can be obtained from the ERATO Overseas Representative or The Royal Society. Applicants must have **a Ph.D. with less than 5 years research experience** or the equivalent, be willing to **commit to at least one year, and be fluent in English or Japanese.** Applications must include **1) a letter describing background and interests, 2) curriculum vitae, 3) list of publications, 4) copies of representative publications, and 5) names and addresses of references.**

**Deadline: 1 February 1993**

**Applications Accepted by**

ERATO Overseas Representative, c/o, ISTA, Inc.
275 Bryn Mawr Avenue, Suite K-46
Bryn Mawr, Pennsylvania 19010 USA
Tel: + 1-215-527-4538, Fax: + 1-215-527-2041
Internet: 71420.3126@compuserve.com

**UK Citizens and Residents Apply Via**

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THE ROYAL SOCIETY
6 Carlton House Terrace
London SW1Y 5AG U.K.
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(W0142)A

# BattelleEurope
## Geneva Research Centers

Battelle is an international technology organization that serves industry and government in generating, applying, and commercializing technology,

In order to develop our research activities in the field of Product Registration and Evaluation, we are seeking a

# SENIOR RESEARCH CHEMIST
## Environmental Fate

Qualifications required for this position are:

- Ph-D degree (or equivalent) in organic chemistry or analytical chemistry
- several years experience in conducting environmental fate studies (soil and sediment metabolism and degradation, soil leaching, photolysis, etc..)
- experience in residue chemistry is desired
- knowledge of GLP
- working fluency in English plus one or two of the major European languages.

This position offers excellent opportunities for personal development, through the confrontation with new problems resulting from our clients' needs, increasing responsibilities in R+D management and an active participation in business development.

**Please address your application, including curriculum vitae and photograph, to the Personnel Manager, BATTELLE EUROPE, Case postale 366, CH-1227 Carouge/Genève.** (W0154)A

# Tepnel Diagnostics PLC

is a rapidly growing Molecular Diagnostic company headquartered in Manchester, England, on the new Science Park.

We are commercialising an exciting range of advanced diagnostics tests for the medical and food industries.

We now require scientists and professionals with specific experience who will appreciate our state of the art facilities and the freedom to develop innovative ideas relating to Tepnel's core aims. We offer above average salaries and benefits together with suitable rewards for loyalty and achievement.

### Deputy to the Group Technical Director

A senior scientific managerial post, the successful candidate will be responsible for the organisation and management of the various projects, reporting directly to the Technical Director.

**Qualifications**

- ***PhD in Biochemistry or Molecular Biology.***
- ***Ten years' experience working with advanced projects and managing to completion.***
- ***Proven laboratory management, project planning and reporting.***
- ***Proven leadership and motivational skills.***

### Project Leaders

Two posts exist for scientists with commercial laboratory experience. You will each be responsible for the day to day running of a team of multi-discipline researchers, of both PhD and graduate levels. The area of science being developed includes DNA chemistry and instrumentation, protein DNA interactions, and molecular modelling.

**Qualifications**

- ***PhD level in Life Sciences.***
- ***5 years experience in a project orientated commercial laboratory.***
- ***Willingness and capacity to manage your own team.***

### Post Doctoral and Graduates

We are assembling a multi discipline team of Biochemists, Molecular Biologists and Instrumentation personnel. Posts now exist for the following specialists.

- **DNA sequencing, DNA handling and data manipulation.**
- **Protein purification, Mono clonal antibody and antibody production experience.**
- **Optical systems and instrumentation.**
- **Biosensor experience of both design and production.**
- **QC/QA experienced specifically in the pharmaceutical industry.**
- **Clinical Microbiological experience.**

**Please send cv and publications to Dr S Minter, Dickinson House, Dickinson Street, Manchester M1 4LF.** (9555)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
LABORATORY OF DEVELOPMENTAL BIOCHEMISTRY

# POSTDOCTORAL SCIENTIST

Applications are invited for a three year post-doctoral appointment to undertake research in a group led by Dr JR tata, FRS, on the regulation of gene expression during post-embroyonic development. The broad area of research concerns molecular and cellular mechanisms underlying tissue-specific expression of genes determining morphogenesis, cell death and tissue remodelling during amphibian metamorphosis. Current studies are focused on the regulation of expression and function of receptors for hormones and morphogens (thyroid hormones, retinoic acid, prolactin), auxiliary transcription factors and response of target genes, both in whole organisms and tissue culture. Previous experience in techniques of recombinant DNA, cell culture and in situ hybridisation is desirable but not essential. The successful candidate will have good opportunities to collaborate with other members of the Laboratory and the Institute.

Starting salary will be in the range £17,575-£26,731 per annum inclusive of London Allowance. MRC Pension Scheme option.

Informal enquiries concerning research projects can be addressed directly to **Dr JR Tata (ext. 2108). Applications including a curriculum vitae, list of publications, and two professional referees should be sent to Mr CR Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, NW7 1AA, not later than 30th October 1992 quoting reference GDBM/0422.**

**THE MRC IS AN EQUAL OPPORTUNITIES EMPLOYER.**

**SMOKING IS ACTIVELY DISCOURAGED.**

MRC Medical Research Council

(9540)A

UNIVERSITY OF BRISTOL
AND
I.A.P.G.R., CAMBRIDGE

# AFRC LINKED RESEARCH GROUP IN REPRODUCTIVE BIOLOGY

The Departments of Biochemistry at the University of Bristol and the Institute of Animal Physiology and Genetics Research offer two 3-year, AFRC-funded Research Assistant posts (one RAIA and one RAIB) to study the structure and function of a rat sperm plasma membrane antigen implicated in sperm-egg recognition.

The postdoctoral position, which will be based in Bristol, will involve cloning and characterising this antigen and subsequently expressing it on the surface of heterologous mammalian cells for egg binding studies. Please quote reference A442.

The RAIB position will be based in Cambridge and will entail antibody production, epitope mapping and IVF studies. Please quote reference A443.

At both locations you will be joining a well established, collaborative team working on a variety of aspects of sperm maturation and function. Salaries will be on the appropriate RAIA and RAIB scales according to age, experience etc.

Informal enquiries may be made to Dr L Hall (Bristol) on (0272) 303504 or Dr R Jones (Cambridge) on (0223) 832312 ext. 311.

For further details telephone Bristol (0272) 256450 (ansaphone after 5pm) or write to the Personnel Office (EO), University of Bristol, Senate House, Bristol, BS8 1TH, quoting the appropriate reference.

The closing date for applications is 29th October 1992.

An Equal Opportunities Employer. (9558)A

UNIVERSITY OF MANCHESTER

# SCHOOL OF BIOLOGICAL SCIENCES

## Plant Metabolism Research Unit

A Postdoctoral Research Associate position is available for 4 years from 1st October, or as soon as possible thereafter, funded under the joint SERC/AFRC initiative in Plant Metabolic Regulation. The project is concerned with the role of intracellular compartmentation in controlling metabolic fluxes. The aim is to produce transgenic plants with altered expression of glucose 6-phosphate dehydrogenase and to determine the impact this has on metabolism. The work will involve enzyme purification, cDNA isolation, plant transformation and biochemical analyses. A technician will be appointed in years 3 and 4 in support of this programme. Experience in biochemistry and/or molecular biology is essential. Salary in the range £12,129 - £14,359 p.a. Further enquiries and applications should be made to Dr. M.J. Emes, Department of Cell and Structural Biology, Williamson Building, University of Manchester, M13 9PL. (Tel. 061 275 3899). Applicants should submit a full c.v. and addresses of two referees by Friday, 30th October, 1992.

*The University is an Equal Opportunity Employer.* (9569)A

I.N.S.E.R.M.

## POSTDOCTORAL POSITION IN MOLECULAR PHARMACOLOGY

Applications are invited for a postdoctoral position available for 1-2 year period beginning between February and September 1992, in an INSERM laboratory working on Molecular and Cellular Pharmacology of antitumor drugs (salary 9.000-12.000 FF/month depending upon experience). Candidates with expertise in molecular biology techniques will be preferred. Fields of interests are molecular and cellular pharmacology of DNA topoisomerase II inhibitors.

Application should be sent with CV to Dr A. JACQUEMIN-SABLON, Unité INSERM 140, INSTITUT GUSTAVE-ROUSSY, rue Camille Desmoulins, 94805 Villejuif, France. FAX: (33) 46.78.41.20. (W0153)A

## FACULTY POSITION

### Immunology of Infectious Diseases

The Department of Microbiology, The University of Texas Health Science Center at San Antonio, has a tenure track faculty position (assistant to full professor) for a person with interest and training in the immunobiology of host-pathogen interactions. Candidates with an accomplished record of research in the area of host immune responses to viruses, bacteria, parasites, or fungi are encouraged to apply. Successful candidates are expected to develop/maintain innovative research programs and to participate in departmental teaching activities. This is an exceptional opportunity to join a strong department committed to excellence in research and teaching and located in a desirable academic-geographic setting. Applicants should mail curriculum vitae, a statement of current and future research goals, and arrange to send three letters of reference by December 31, 1992 to **Dr. Judy M. Teale, Chair, Immunopathogenesis Search Committee, Department of Microbiology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7758.** *UTHSCSA is an equal opportunity, affirmative action employer.* (NW8482)A

## FACULTY POSITION
## YEAST MOLECULAR GENETICS
### Department of Biology
### Syracuse University

Applications for a tenure-track position are invited from individuals with doctoral training and postdoctoral research experience in molecular genetics who will use yeast as an experimental organism in their research. This appointment is contingent on final administrative approval and is part of a continuing expansion within the area of Cell and Molecular Biology in the Syracuse University Biology Department. Appointment is most likely to be made at the Assistant Professor level, but more senior candidates are also welcome to apply. The successful candidate will carry out an independent research program and participate in teaching of undergraduate and graduate students. Applications should include curriculum vitae, a brief description of proposed research and the names of three scientists willing to write letters of recommendation. Applications should be sent by December 15, 1992 to:

**Chair of Yeast Molecular Biology Search Committee, Lyman Hall, 108 College Place, Syracuse University, Syracuse, NY 13244-1270.**

*Syracuse University is an Equal Opportunity/Affirmative Action Employer; women and members of all ethnic groups are expecially encouraged to apply.* (NW8471)A

THE UNIVERSITY OF LEEDS

Centre for Plant Biochemistry and Biotechnology

## MOLECULAR BIOLOGIST

### Sex Determination in Plants

Applications are invited for a postdoctoral Fellow to work on the molecular basis of XY chromosome mediated sex-determination in plants. The work will involve the construction and screening of cDNA and genomic libraries to isolate male specific genes from dioecious white campion. The successful candidate will require a PhD in a relevant field and have expertise in a broad range of molecular biological techniques. The position, funded through the AFRC Plant Molecular Biology Initiative, is available for a fixed period of up to three years and will be held in the Centre for Plant Biochemistry and Biotechnology, a multidisciplinary Research Centre within the Departments of Biochemistry & Molecular Biology and Pure & Applied Biology.

Salary within the range (£12,129 — £19,328) on the IA scale for Research Staff (pay award pending), according to qualifications and relevant experience.

Informal enquiries may be made to Dr Phil Gilmartin (tel: 0532 332902). Further details of this and other positions available within the Centre, may be obtained from Dr Andrew MacGregor (tel: 0532 332863).

**Application forms and further particulars may be obtained from and completed applications forwarded to the Academic Staff Office, The University of Leeds, Leeds LS2 9JT, (tel: 0532 335771 — direct line) quoting reference no. 83/126.**

Closing date for applications: 1 November 1992.

*The University of Leeds promotes an equal opportunities policy.* (9513)A

THE UNIVERSITY OF SHEFFIELD

## LECTURESHIP IN ORGANIC CHEMISTRY

Applications are invited from those with interests and expertise in any aspect of organic chemistry.

Informal enquiries to the Head of the Department, Professor CJM Stirling FRS, (Tel: 0742 768555, ext. 4462).

Further details of the appointment and of the Department from **Director of Personnel Services, The University, PO Box 594, Firth Court, Western Bank, Sheffield S10 2UH (Tel: 0742 768555, ext. 4144), to whom applications including a full CV and the names/addresses of three referees (3 copies of all documents), should be sent by 1 December 1992. Ref: R199.** *An Equal Opportunity Employer* (9535)A

**PUBLIC HEALTH LABORATORY SERVICE BOARD**

COMMUNICABLE DISEASE SURVEILLANCE CENTRE AND THE CENTRAL PUBLIC HEALTH LABORATORY

# DEPUTY EDITOR/ MICROBIOLOGIST

Experienced clinical microbiologists with a higher degree are invited to apply for the post of Deputy Editor in the Editoral Section of the Communicable Disease Surveillance Centre (CDSC). Responsibilities will include editing the microbiological component of the weekly **Communicable Disease Report** (CDR) and providing microbiological expertise for other publications, including the four-weekly CDR Review. This appointment is for two years in the first instance and will provide the opportunity to review and develop the content, style and format of the weekly CDR in conjunction with the Medical Editor. The post-holder will also make a scientific contribution to the study of nosocomial disease (in association with the Division of Hospital and Respiratory Infection of CPHL) with a view to enhancing surveillance and information output.

Candidates should possess a first or second class honours degree in a biological subject of relevance to microbiology; a higher degree; several years' postgraduate experience in clinical microbiology, and an interest in epidemiology. Candidates will be expected to have published original work in learned journals; to have a flair for writing and editing, and the ability to communicate well with colleagues in other disciplines. The successful candidate will be appointed to the Clinical Scientist Scale, Grade B (17-19). Current salary scale: £21,947 to £23,738 (plus £1,144 London Weighting allowance).

Those interested may contact the Medical Editor, Dr. Gordon Reeves, for further information (telephone 081 200 6868, ext 4488). A job description and application form may be obtained from the **Personnel Department, Public Health Laboratory Service Board Headquarters, 61 Colindale Avenue, London NW9 5DF (tel. 081-200 1295, ext 3690).**

Closing date Friday 30th October 1992.

We are an Equal Opportunity Employer. (9544)A

**ELSEVIER SCIENCE PUBLISHERS LTD**

# EDITOR IMMUNOLOGY TODAY

Elsevier Science Publishers Ltd is the British company of the international Elsevier Science Publishers Group, one of the world's largest scientific, technical and medical publishers. Its Cambridge-based division, Elsevier Trends Journals, is seeking an Editor for its monthly review journal Immunology Today, now in its thirteenth year of publication and one of the most highly cited titles in biomedicine. The journal publishes commissioned reviews, commentaries and reports concerning all aspects of the immune system and its interactions with infectious agents, and is particularly noted for its coverage of new ideas and hypotheses.

The successful applicant will have a thorough grasp of the major areas of research in immunology and related fields, and be able to commission successfully in these areas through developing a clear appreciation of the needs of the readership, and through the ability to communicate directly with scientists at the forefront of developments. Ideally, candidates will have a post-graduate qualification in a relevant field, and although not a prerequisite, some publishing experience would be desirable. An excellent command of written English, self motivation and a willingness to learn new skills, especially related to the commercial aspects of publishing, are essential.

An attractive salary will be offered to the successful applicant, and other benefits include 25 days holiday and contributory pension scheme. Assistance with relocation will be considered where appropriate.

To apply, send a written letter of application accompanied by a full cv to: **Dr David Bousfield, Publisher, Elsevier Trends Journals, 68 Hills Road, Cambridge CB2 1LA, UK (Tel: 0223 315961; Fax 0223 464430). Applications to arrive no later than 30th October, 1992.**

NO AGENCIES (9571)A

THE UNIVERSITY OF SHEFFIELD

Robert Hill Institute and Department of Animal & Plant Sciences

# POSTDOCTORAL RESEARCH POSITIONS IN PLANT MOLECULAR BIOLOGY

Applications are invited for two positions, tenable from 1 January 1992 for three years and funded by the joint AFRC/SERC programme on the Biochemisty of Metabolic Regulation in Plants. Both positions are concerned with transformation of plants in order to study the control of metabolism.

One position is to study the control of gluconeogenesis by purifying cucumber phosphoenolpyruvate carboxykinase, isolating cDNA and transforming plants. Informal enquiries to Dr R C Leegood (0742-768555 ext 6404)

The other position is concerned with the genetic manipulation of carbohydrate metabolism by the transformation of plants of *Arabidopsis thaliana* with sucrose synthase and sucrose phosphate synthase. Informal enquiries to Dr W P Quick or Dr S A Rolfe (ext 4115).

**Salary in the range £12,129-£14,936 pa (under review), according to age and experience. Further details from Director of Personnel Services, The University, PO Box 594, Firth Court, Western Bank, Sheffield S10 2UH (tel: 0742-768555 ext 4144), to whom applications including a full CV and the names/addresses of three referees (3 copies of all documents) should be sent by 16 November, 1992. Please indicate if you are applying for both posts. Ref: B2060.**

*An equal opportunity employer.* (9563)A

**MEDICAL RESEARCH COUNCIL**
**CELL MUTATION UNIT**
**UNIVERSITY OF SUSSEX**

# Postdoctoral Position in Photoimmunology

A 2-year postdoctoral position is available in an EC-funded project to study the DNA damaging effects of sunlight and UV-B on cells of the immune system. The work may be relevant to mechanisms of carcinogenesis and immunosuppression.

The MRC Cell Mutation Unit has extensive experience in the fields of DNA damage and repair. The successful candidate is likely to have experience in immunology and mammalian cell culture.

The post is likely to be on the MRC non-clinical scientist Grade II scale (currently £15,530-£18,540).

For further information phone Dr Colin Arlett or Dr Michael Green (0273 678119).

Applications, including a CV and addresses of two referees should be sent to **Mrs Margaret Bunn, MRC Cell Mutation Unit, Sussex University, Falmer, Brighton BN1 9RR, UK** by 13th November 1992. (9552)A

MRC Medical Research Council

MRC Medical Research Council

**Medical Research Council**
**Reproductive Biology Unit, Edinburgh**

# CAREER APPOINTMENTS

The Unit is seeking to appoint two scientists to study, respectively 1) the development of the neuroendocrine system of the fetus, and 2) the endocrine and paracrine steroid regulation of gene expression in the testis. Applicants should have postdoctoral experience, an interest in the regulation of reproductive processes and an established record of high quality research. Appointments will be made within the Non-clinical Scientific Grade, with tenure where applicable, and with effect from October 1993. Current salary £15,533-£24,689. Applications, including a CV, the names of two referees and a 2000 word statement of research interests should be sent to Professor DW Lincoln, Director, MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW by 16th October 1992. Phone: 031 229 2575 (Miss Ena McKay) for further particulars. The MRC is an equal opportunity employer. (9553)A

**The Medical College of St. Bartholomew's Hospital**
**DEPARTMENT OF IMMUNOLOGY**

# Post/Doctoral Research Fellow

A Post/Doctoral Research Fellow is required to join a small team working on the role of auto-immunity in the pathology of HIV infection. Experience in the production of monoclonal antibodies is essential and a knowledge of ELISA and Western blotting would be advantageous.

The salary is on the Research scale 1A (£14,263-£21,462pa inclusive).

For further information please contact **Dr WJW Morrow on 071-601 8428. Applications in the form of a full CV and the names of two professional referees should be sent to the Department of Immunology, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE by 30 October 1992.**

*Working towards Equal Opportunities.* (9547)A

# UNIVERSITY OF OXFORD

## OXFORD CENTRE FOR MOLECULAR SCIENCES

## POSTDOCTORAL RESEARCH ASSISTANTS

A postdoctoral chemist is required to work on the synthesis of potential inhibitors of phosphorylase with Dr G W J Fleet. The work will involve the synthesis of relatively small molecules and experience in general synthetic organic chemistry and/or carbohydrate chemistry would be an advantage. The post is available from 4 January 1993 and is for 6 months in the first instance (Ref GWJF).

A second postdoctoral position is available in the group of Dr C M Dobson, investigating the molecular basis of protein folding. The project involves the structural characterisation of folding intermediates by protein chemical methods and biophysical techniques, including NMR and CD. Experience in the purification and handling of proteins would be an advantage. The post is available from 4 January 1993 and is for 21 months in the first instance (Ref CMD).

**Both posts are funded by SERC/MRC on the RA1A scale £12,129-19,328 according to age and experience. Applicants should send a full curriculum vitae and the names and addresses of two referees, to the Administrator, OCMS, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, quoting the relevant reference. Closing date for application Friday 16 October 1992.** (9561)A

The University is an Equal Opportunity Employer

# ASSISTANT PROFESSOR

### *Immunobiology of Infectious Diseases and Viral or Bacterial Pathogens*

### University of North Carolina at Chapel Hill

Applications are invited for a tenure track position to complement existing strength in molecular and cellular immunology, immunopathology, virology, bacteriology, microbial pathogenesis, and cellular and molecular genetics. A doctoral degree and relevant postdoctoral experience are required. The candidate will be expected to lead a vibrant and independent research program, and will participate in graduate/medical teaching. Preference will be given to applicants who reply by December 1, 1992. Minority and female candidates are asked to voluntarily identify themselves.

**Interested candidates should submit curriculum vitae, description of current and future research goals, representative reprints and names/addresses of three references to: Dr Jenny Ting, Chair, Search Committee, Department of Microbiology and Immunology, CB# 7290, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7290.**

*The University of North Carolina is an affirmative action/equal opportunity employer.* (NW8473)A

INSTITUTE OF OPHTHALMOLOGY
(University of London)
Bath Street, London EC1V 9EL

# RESEARCH ASSISTANT

A Research Assistant is required for a research project into the modulation of ocular wound healing with antiproliferative agents and growth factors funded by the International Glaucoma Association. This project will involve cell culture, Immunostaining, molecular techniques and growth factor/growth factor receptor assays. Previous experience in these techniques would be an advantage but not essential. The appointment will be for one year with possible renewal, subject to funding, for two further years.

Fixed salary at a point in the range: £14,171-£15,653pa (inc LA) according to experience and qualifications.

Informal enquiries welcome, please ring Dr Peng Khaw, 071-608 6803.

For further information and an application form, please write to Louise Flatten at the above address, enclosing an addressed envelope (no stamp necessary) and quoting the reference 109325. Closing date for receipt of completed application forms: 15 October, 1992. (9543)A

## UNIVERSITY OF CAMBRIDGE
## Institute of Biotechnology

## POSTDOCTORAL RESEARCH ASSISTANTSHIP

### *Biological Applications of Scanning Probe Microscopy*

Applications are invited for a SERC-funded postdoctoral research assistantship to study the immobilisation of enzymes and immunoglobulins to solid surfaces of interest in biosensors, solid-phase immunoassay, biocompatibility and liquid chromatography. The appointee will be expected to apply the Institute's TopoMetrix TMX 2000 scanning tunnelling and atomic force microscope and other sophisticated biophysical techniques to this study.

The post is available immediately. Applicants ideally should have experience of scanning probe techniques and/or other physical techniques for the study of interfacial biochemistry and hold, or expect to receive shortly, a PhD relevant to these. Salary will be on the Research Assistant 1A salary scale.

Applicants should submit a *curriculum vitae*, including the names and addresses of three referees by 16 November 1992 to **Dr C R Lowe, Institute of Biotechnology, Tennis Court Road, University of Cambridge CB2 1QT from whom informal enquiries may be made. (Tel: 0223 334160).** (9567)A

## THE UNIVERSITY OF BRITISH COLUMBIA
## Faculty Position in Astronomy/ Astrophysics

Applications are invited for a tenure track assistant position in astronomy beginning July 1, 1993. Appointment may be considered at a higher rank for a woman with exceptional qualifications. Applications are encouraged in both observational and theoretical astrophysics; we are seeking an outstanding scientist who will complement our existing strengths in stellar, galactic and extragalactic astronomy, cosmology and astronomical instrumentation. The appointee will be expected to teach undergraduate and graduate courses, to develop a strong research program, and to supervise graduate students. Salary will be commensurate with experience. Applicants must possess a Ph.D. This position is subject to final budgetary approval. *In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. UBC encourages qualified women and minority applicants.* Applications including a resume, a statement of research interests, and the names of three referees should be sent to **Dr. R. M. Ellis, Head, Department of Geophysics and Astronomy, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4, by December 1, 1992.** (NW8460)A

# MICROBIOLOGIST

## FACULTY POSITION in the MOLECULAR AND CELLULAR BIOLOGY DEPARTMENT

### at the UNIVERSITY OF ARIZONA

As part of a continuing expansion, the Department of Molecular and Cellular Biology, in collaboration with the new interdepartmental Undergraduate Microbiology Program, has an opening for a new tenure track faculty member for appointment in 1993. We are seeking candidates with a broad interest in molecular approaches to understanding both basic and applied problems related to the physiology, development, regulation or genetics of prokaryotic systems. This faculty member will be responsible for teaching undergraduate bacterial physiology as part of the Undergraduate Microbiology Program. Outstanding candidates at either the Assistant or Associate Professor level are encouraged to apply. Successful candidates will be expected to establish a competitive research program and teach undergraduate and graduate students. Send curriculum vitae, a 1-2 page statement of research interests, and have at least three supporting letters sent to: **Chairman, Microbiology Search Committee, Department of Molecular and Cellular Biology, Life Sciences South Building, Room 444, University of Arizona, Tucson, AZ 85721, USA.** To ensure consideration, applications should be received by **November 20, 1992,** although applications will be accepted until the position is filled. *The University of Arizona is an Equal Opportunity Affirmative Action Employer. Women and minorities are encouraged to apply.* (NW8481)A

## POSTDOCTORAL RESEARCH POSITIONS IN MOLECULAR BIOLOGY/ IMMUNOLOGY

e available to study the (i) the immunopathology / immunoregulation of rheumatoid joint diseases in humans and in an experimental autoimmune animal model or (ii) the pathological bone resorption in loosened joint replacements.

The candidate(s) must have skills in at least one of the following areas: (1) molecular biology techniques, (2) cell and tissue culture work including radioisotope techniques, (3) basic immunological/immunochemical techniques or (4) basic biochemical methods (preparative and analytical methods, amino acid sequencing, electrophoresis, etc.).

*Enquiries and applications should be directed to*

**Dr. Tibor T. Glant, Department of Biochemistry, Rush Medical Center, 1653 West Congress Parkway, Chicago, IL, USA. Phone: (312) 942-5855; Fax: (312) 942-2101.**

(NW8443)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA

HIGHER SCIENTIFIC OFFICER (Band 1)
MOLECULAR BIOLOGIST
(3 year appointment)
ZOOLOGY DEPARTMENT

A Molecular Biologist is needed to expand studies on the molecular biology of plant parasitic nematodes. The project will be initially for 3 years and will concentrate on studying within species variation in mitochondrial DNA in relation to biological variation. The successful candidate will have substantial research experience in many aspects of molecular biology and experience of working on invertebrates could be useful. The appointee will work closely with nematologists and will be expected to help supervise a Ph.D. student funded by an E.C. grant. Encouragement will be given to expanding the project and to efforts to attract further external funding.

Salary: £12,339-£17,227.

Qualifications: Candidates should normally have a first or upper second class honours degree in an appropriate scientific, or related subject, together with at least 2 years' relevant postgraduate research or other relevant experience.

Non-contributory superannuation.

The Institute is an equal opportunities employer.

The Scottish Crop Research Institute is grant-aided by the Scottish Office Agricultural and Fisheries Department (SOAFD).

Curriculum vitae complete with the names and addresses of three referees should be sent to the Personnel Officer by Friday 16th October 1992 quoting the appropriate reference (Zoo/6/92). (9550)A

## SENIOR MEDICAL STATISTICIAN

THE BEAUFOUR-IPSEN GROUP,
A RAPIDLY EXPANDING AND DYNAMIC
FRENCH OWNED INTERNATIONAL
PHARMACEUTICAL GROUP WITH
A TURNOVER OF OVER ECU 315 MILLION
(£ 220 MILLION) IN 1991, IS SEEKING
A SENIOR STATISTICIAN
TO ASSIST IN THE GROWTH
OF ITS BIOMETRY UNIT.

Within the Biometry Unit of the Central Development Department, you will be expected to take on the following responsibilities in the area of clinical trials :

- thorough supervision of statistical methodology and monitoring of the reports carried out in the Group's subsidiaries ;
- heading the statistics unit, elaborating statistical protocols, verification and validation of reports, as well as liaison with subcontractors.

Ideally, the successful candidate should be a qualified statistician (PhD or MSc) with around five or more years' experience in applying statistics and the analysis of clinical trials, preferably within the pharmaceutical industry. A thorough understanding of SAS is required.

A sense of autonomy, team spirit and persuasiveness are expected, in order to ensure a permanent and constructive dialogue with your partners in France and abroad.

A fair command of French would be useful, although adequate training will be provided.

A medical background (Dr in Medecine) would be welcome, though it is not compulsory.

The position is located in central Paris, with the opportunity for frequent travel (Europe and USA).

Attractive pay package and working conditions are offered.

Please write enclosing a C.V. and quoting ref. SMS, to :
Human Resources Department,
INSTITUT HENRI BEAUFOUR
42, rue du Docteur Blanche - 75016 PARIS - FRANCE

(For further details, please telephone : 010 33 1 44 30 43 43)

MEDIAPA (W0159)A

## RESEARCH FELLOW/STAFF SCIENTIST

A position as a postdoctoral research fellow or staff scientist is available immediately to participate in the research program utilizing the PANURGE ion microprobe. The position involves substantial responsibility overseeing the day-to-day operation of the ion probe and offers the opportunity for creative research in a wide range of subjects in cosmochemistry and geochemistry, including the isotopic and chemical properties of meteorites and interstellar grains and the trace element geochemistry of natural and synthetic silicates and oxides. A major effort to characterize diffusion transport of elements and of volatile compounds in minerals and glasses is also underway. Individuals with a strong background in isotope geochemistry and/or experimental analysis are encouraged to apply. While previous experience with an ion microprobe is desirable it is not required. We will provide training. Candidates should have a strong interest in methods and principles of instrumental analysis and knowledge of some mineralogy. Applicants should have a Ph.D. in analytical chemistry, geochemistry, petrology or experimental physics. Level and salary are dependent on experience.

Send resume and names of three references to **Prof. G.J. Wasserburg, Division of Geological and Planetary Sciences 170-25, California Institute of Technology, Pasadena, CA 91125 FAX: 818-568-0935; E-Mail; kathie@legs.gps.caltech.edu.**

*Caltech is an equal opportunity/affirmative action employer.*

(NW8477)A

**Departments of Biochemistry and Botany**

## TWO POSTDOCTORAL POSITIONS IN PLANT MOLECULAR SCIENCE

UNIVERSITY of GLASGOW

One position, supported by the AFRC/SERC plant metabolism initiative, is to investigate the regulation of PEP carboxylase by reversible phosphorylation in $C_4$ plants. The other, funded under the AFRC plant molecular biology programme, is to use molecular biology in a study of the role of PEP carboxylase kinase in circadian rhythms in *Bryophyllum*. Applications are invited from candidates with experience in enzymology, plant biochemistry or molecular biology, who should have or will shortly obtain a PhD in an appropriate subject. The successful candidates will join an active and well funded group taking a multi-disciplinary approach to plant science. Both positions are available for up to three years from 1 October 1992 but candidates who would wish to start later will also be considered. Salaries will be at the appropriate point on Scale 1A.

**Informal enquiries may be made to Dr HG Nimmo (041 339 8855 ext. 4721) to whom applications, with the names of two academic referees, should be sent as soon as possible at the Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.** (9545)A

UNIVERSITY OF DUBLIN

TRINITY COLLEGE

## GENOME SEQUENCING AT THE NATIONAL PHARMACEUTICAL BIOTECHNOLOGY CENTRE TRINITY COLLEGE DUBLIN

An automated DNA sequencing facility is being established at Trinity College. Applications are invited for the position of Manager of this facility. The successful applicant will liaise closely with groups within the Department of Genetics involved in whole genome sequencing projects (*Bacillus*, yeast, *Arabidopsis*) and will operate an Applied Biosystems Model 373A DNA sequencing machine, manage and analyse DNA sequences. The appointment will be for a period of two years and will be made at any level up to postdoctoral. Remuneration and exact duties will depend on the experience of the successful applicant. A background in molecular biology is essential and experience of sequencing and computing is desirable.

Further information may be obtained from Dr. Kevin M Devine, Department of Genetics, Trinity College, Dublin 2 Ireland. Tel: 353-1-7021872; FAX: 353-1-6798558; E-Mail: KDEVINE@VAX1.TCD.IE.

**Trinity College is an Equal Opportunities Employer.** (9528)A

## ZOOLOGY INSTITUTE UNIVERSITY OF BASLE SWITZERLAND

### ONE ASSISTANT POSITION

Is foreseen for 1 October 1993 in Population Biology. Successful candidates will have used evolutionary insights to solve problems in genetics, ecology, or behaviour. The person appointed will teach evolutionary genetics, but research in this area is not required. Quality of research and fit to the existing group are more important than the question asked or organism used. We try to balance field and laboratory, theory and experiment within the group.

A PhD, postdoctoral experience, and the ability to teach in German are required. The decision will be made by 1 March 1993, leaving time to study German before teaching begins in November 1993. The successful candidate will direct an independent research programme, seek grant support, direct MSc and PhD students, offer an advanced seminar in the area of expertise, and take part in the teaching of the biology curriculum. Teaching can be concentrated in one semester, leaving the rest of the year free for research.

The term of appointment will be 5 years. The salary will be in the range Sfr 60-75'000. Habilitation will be possible. Please send a curriculum vitae, reprints of 3-4 papers, and the names of two references by 15 January 1993 to: Professor S C Stearns, Zoologisches Institut, Universität Basel, Rheinsprung 9, CH-4051 Basel, Switzerland. (W0165)A



## THE AUSTRALIAN NATIONAL UNIVERSITY

## SENIOR FELLOW/FELLOW

### IN THE DEPARTMENT OF BIOGEOGRAPHY & GEOMORPHOLOGY

***Division of Society & Environment***

Research School of Pacific Studies

Applications are invited for the position of geomorphologist in the Department of Biogeography & Geomorphology at the Research School of Pacific Studies, at the level of Fellow/Senior Fellow, continuing. Appointment is for 5 years initially, with the possibility following review, of re-appointment to geomorphology is essential, and superior skills are sought in either the analysis of young sedimentary deposits, or in pedology including palaeopedology, or both. The succesful applicant may expect to assume responsibility for research projects concerned with geomorphologic changes, including land degradation, in relation to climatic factors and anthropogenic effects throughout the period of human presence in the western Pacific region.

Further particulars: Please obtain these before applying from Appointments (41073), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, or from the School Secretary, Research School of Pacific Studies, Australian National University (tel. (61 6) 249 2678 or Fax (61 6) 257 1893).

Closing date: 6 November 1992. Ref: PA 17.9.1.

SALARY: Fellow (Academic Level C) A$50,225-A$57,913 pa; Senior Fellow (Academic Level D) A$57,913-A$66,625 pa.

APPLICATIONS addressing the selection criteria should be submitted in duplicate to the Secretary, The Australian National University, G.P.O. Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University has a "no-smoking" policy in all university buildings and vehicles.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W0163)A

## UNIVERSITY OF NOTTINGHAM

### APPLIED BIOCHEMISTRY AND FOOD SCIENCES

## RESEARCH ASSISTANT (1A/1B)

Applications are invited for a research assistant to work on an AFRC funded project to study the effects of genetic engineering on the processing qualities of tomato fruit. The work will involve the extraction and biochemical analysis of enzymes and cell wall carbohydrates along with the analysis of processed tomato products by a range of visciometric and microscopy techniques. The project will be jointly supervised by Dr J. Mitchell and Dr G. Tucker.

Candidates should have at least a first degree in a biologically related subject and preferably have a good understanding of chemistry and physics. The post is for a period of three years starting as soon as possible. The appointment will be made at either the postgraduate or postdoctoral level according to qualifications. A postgraduate appointee would have the option to register for a Ph.D.

Applicatons along with the names and addresses of two referees should be sent to: **Dr G. Tucker, University of Nottingham, Applied Biochemistry and Food Sciences, Sutton Bonington Campus, Loughborough, Leics. LE12 5RD.** The closing date for applications is October 12th. Salary, depending on age and experience, in the range of £12,129 — £15,688.

(9533)A

THE STATE UNIVERSITY OF NEW JERSEY

# RUTGERS

## FACULTY POSITION

### DEVELOPMENTAL GENETICIST

Applications are invited for a tenure-track faculty position at the Waksman Institute with a joint appointment in the Department of Molecular Biology and Biochemistry. The Waksman Institute has been at the center of a major initiative to transform Rutgers University and the adjoining University of Medicine and Dentistry of New Jersey into a major, world class research center. Nearly 100 new faculty members in the biomedical area alone have been recruited during the past seven years. The Waksman Institute has added 11 members during this period with emphasis on four areas; molecular developmental genetics, microbial genetics, plant molecular genetics, and structural biology. Candidates are sought to further expand the developmental genetics group. The successful applicant will be expected to conduct a vigorous independent research program and to participate in instructional activities. Recently renovated laboratory space and generous start-up funds are available.

**Candidates should send a biographical sketch (with a list of publications and a description of research interests) and request that three letters of reference be sent to: Dr Joachim Messing, Director, The Waksman Institute, Rutgers, The State University of New Jersey, PO Box 759, Piscataway, NJ 08855-0759.**

*An affirmative action/equal opportunity employer.* (NW8476)A

## Centro Internacional de Agricultura Tropical (CIAT)

## Head of Genetic Resource Unit

CIAT's Genetic Resources Unit cares for the world's largest collection of *Phaseolus vulgaris, Manihot* spp., and selected forages for acid soils. The Unit's Head (1) supervises its activities, facilities, and staff of 50; (2) contributes within his/her speciality; and (3) coordinates and facilitates the collection, characterization, documentation, conservation, multiplication, and distribution of germplasm.

The sought-for candidate has a Ph.D. and considerable experience in germplasm management or related field. Administrative ability and service attitude are essential; Spanish and/or Portuguese an advantage.

Salary and benefits are comparable with other international organizations. Submit curriculum vitae and names and addresses of three referees by 17 November to: **Director General, CIAT, Apartado Aéreo 6713, Cali, Colombia.**

(W0156)A

## EVOLUTIONARY ANTHROPOLOGIST/BIOLOGIST
## EMORY UNIVERSITY
### Departments of Anthropology and Biology

announce a joint, tenure track, assistant professor position for an evolutionary anthropologist/biologist. Preference will be given to anthropologists or biologists with active research programs on the genetics, molecular evolution, or behavioral ecology of human populations. Candidates must be able to interact effectively with faculty in both Anthropology and Biology programs, have a strong research record, and be committed to quality teaching at the undergraduate as well as the graduate level in both Anthropology and Biology departments.

Application deadline is December 31, 1992. Send *curriculum vitae*, description of current and planned research and teaching interests, and the names of four referees to: **Dr. Carol M Worthman or Dr. Mark Ridley, Search Committee Chairs, Department of Anthropology, Emory University, Atlanta, GA 30322.**

*Emory University is an Equal Opportunity/Affirmative Action Employer.* (NW8474)A

## POSTDOCTORAL POSITION
### SHRINERS HOSPITAL
*Affiliated with McGill University*

Supported for two years by the Medical Research Council of Canada, this position is available immediately to clone the gene encoding a novel transcription factor expressed during neuronal differentiation. The protein has already been purified using DNA affinity chromatography. Applicants should have a PhD in biochemistry or related fields. Experience with cDNA cloning preferable.

Please forward your curriculum vitae, a brief statement of research interests, and two letters of reference to Dr René St-Arnaud, Genetics Unit, Shriners Hospital for Crippled Children, 1529 Cedar Avenue, Montréal, Québec, Canada H3G IA6. Fax: (514) 842-5581. (NW8465)A

## University of Vermont
### Department of Medicine
### Postdoctoral Research Associate

To work on studies on the role of energy expenditure in the development of obesity in children. Experience with human nutrition research techniques highly desirable. Send applications (CV and 3 letters of recommendation) or informal enquiries to: **Michael I Goran PhD, Department of Medicine, Metabolic Unit, University of Vermont, Burlington, Vermont 05405. (Tel: 802-656-2438, FAX: 802-656-5371).** *Application deadline: November 15th, 1992.* (NW8483)A

## Centre d'Etude du Polymorphism Humain (C.E.P.H.), Paris
## THREE POST-DOCTORAL RESEARCH FELLOWS

to develop new techniques in large scale DNA sequencing, or to participate in ongoing projects searching for the human genes involved in Diabetes and Aging.

Contact:

**Professor Daniel Cohen;**
**CEPH;**
**27, rue Juliette Dodu;**
**75010 PARIS (France).** (W0162)A

## UNIVERSITY OF CAMBRIDGE
### Department of Plant Sciences
### PLANT MOLECULAR BIOLOGY

Applications are invited for a postdoctoral research assistant IA to work with Dr J C Gray on DNA-binding proteins of the nuclear chromosomal scaffold in higher plants. Experience of molecular biology techniques is essential. The post, which is supported by SERC, is for a period of 3 years from 1 January 1993.

**Applications, including CV and the names and addresses of two referees should be sent to Dr J C Gray, Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK (tel 0223 333925) not later than 1 November 1992.** (9562)A

## UNIVERSITY OF GLASGOW
### Department of Medicine and Therapeutics/ Department of Genetics
### Post-Doctoral Research Assistant
**(Salary £12,129 p.a.)**

UNIVERSITY of GLASGOW

This is a three year post funded by the British Heart Foundation to work with Drs A F Dominiczak (Dept. of Medicine) and Dr P G Sutcliffe (Dept of Genetics) on the project: "Cardiovascular phenotypes and genetic linkage analysis in hypertension". The work involves the analysis of molecular genetic markers (microsatellite polymorphism), genetic linkage analysis as well as physiological measurements such as blood pressure and vascular smooth muscle characteristics in cell culture. You should have experience in the use of standard molecular genetic techniques and an interest in cardiovascular physiology.

The proposed starting date is November 1, 1992 (negotiable).

**Applications with names and telephone/fax numbers of two referees should be made to Dr A F Dominiczak, Dept. of Medicine and Therapeutics, Western Infirmary, Glasgow, G11 6NT. Tel: 041 339 8822, ext. 4688. Fax 041 339 1263.** (9529)A

### THE UNIVERSITY OF BIRMINGHAM
### SCHOOL OF PHYSICS AND SPACE RESEARCH
## POSTDOCTORAL RESEARCH FELLOW

Required to join an active SOLAR PHYSICS GROUP to work on a joint international coronagraph for the SOHO spacecraft. There will be an opportunity to work on data analysis and interpretation from an existing, current database from other space instruments in areas relevant to the anticipated science from the coronagraph.

Informal enquiries to Dr GM Simnett on 021 414 6469.

Salary on a range £12,129-£19,328 (under review) for three years.

**Application forms (3 copies returnable by 26th October 1992) and further particulars available from The Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT, telephone 021 414 6483 (24 hours), quoting reference S13235/92A.**

*Working towards equal opportunities.* (9554)A

## POSTDOCTORAL POSITIONS

available to study cell and molecular biology of insulin and growth factor action. Emphasis on regulation of tyrosine kinase and phosphatase functions. Experience with protein purification/enzymology, recombinant DNA, and/or mammalian expression systems desired. Start Nov. 1/92 and thereafter. Send CV and three references to: **Dr. Barry I. Posner, Chief of Endocrinology, Polypeptide Hormone Lab., Royal Victoria Hospital, 687 Pine Avenue West, Room H5.45, Montreal, Quebec H3A 1A1.** Canadian Immigration requires priority to be given to permanent residents of Canada. (NW8468)A

## IMPERIAL COLLEGE
of Science, Technology & Medicine

Department of Chemistry
## POSTDOCTORAL POSITION
### Stress Protein Chemistry

Applications are invited for an SERC-funded postdoctoral position (RA 1A) to work on the structure, function and chemistry of stress proteins. The successful applicant will work in a multidisciplinary research group applying the techniques of biological nmr, enzyme kinetics, protein biochemsitry, molecular biology and organic synthesis to problems in stress protein chemistry. A background in nmr and protein purification would be helpful.

The position is available for 3 years from 1 October 1992. Applications, including a CV and the names of two referees, should be sent as soon as possible and no later than 1 November 1992, to: **Dr AD Miller, Imperial College of Science Technology and Medicine, Department of Chemistry, South Kensington, London SW7 2AY. Tel: 071-225 8331; Fax: 071-589 3869.** (9570)A

## School of Biological Sciences
## Department of Biochemistry

Applications are invited for an academic position in the Department of Biochemistry. The successful candidate will be expected to strengthen the current research activities in the SERC-supported Centre of Molecular Recognition of the University, which, broadly, studies biological problems at the molecular level. Candidates should have an active research programme and an appointment will be made at the level of Lecturer, Senior Lecturer or Reader, depending on age, experience and track record in research.

Salary will be either on the Lecturer Grade A (£12,860-£17,827 per annum) or B (£18,572-£23,739 per annum) level, or on the Senior Lecturer and Reader scale (£24,922-£28,165 per annum).

Further particulars are available from the Personnel Department (S), University of Southampton, Highfield, Southampton SO9 5NH (0703 593801) to whom applications (2 copies of curriculum vitae, the names, addresses and telephone numbers of 2 referees and 2 copies of all correspondence relating to your application) should be sent to arrive no later than 12 November 1992. Please quote reference S/69/NA.

**Working for Equal Opportunities.** (9574)A

**University of Southampton**

## THE UNIVERSITY OF OXFORD
***The Nuffield Department of Surgery***
***The John Radcliffe Hospital***
***Headington, Oxford OX3 9DU***

## Genetic-pathophysiology of Autoimmune Disease

A Postdoctoral research position is available immediately in this department for a suitably qualified scientist holding a PhD or equivalent in Genetics, Immunology or Molecular Biology. The successful candidate will join a young and enthusiastic group working under the direction of Dr John Todd to characterise the function of genes that cause the autoimmune disease, Insulin Dependent Diabetes (*Nature* **351,** 542 & **353,** 262, 1991; *Nature Genetics,* in press; *Diabetes* **41,** 1029, 1992).

This position is supported by a three year grant (Wellcome Trust) with the salary being based upon the University RS1A grade.

Salary scale RS1A £12,129-£19,328 (under review).

*Further details may be obtained from Dr John Todd. Tel: (0865) 220145, Fax (0865) 65063.*

**Formal applications in writing together with a full CV and the names, addresses and telephone/fax numbers of two academic referees, should be sent to the Administrator at the above address.**

Closing date: 3rd November 1992.

The University of Oxford is an equal opportunities employer. (9576)A

UNIVERSITY OF OXFORD
Research Laboratory for Archaeology

## POST-DOCTORAL RESEARCH ASSISTANT
### IN ARCHAEOLOGICAL SCIENCE

Applications are invited for a PDRA to work with Dr R E M Hedges (Deputy Director). The position is for five years in the first instance. Applicants from a broad range of backgrounds relevant to archaeological science will be considered, but preference will be given to those with experience and interest in organic or biological areas of molecular science. Further details on this post are available on enquiry.

Applicants should have an honours degree, and a PhD or equivalent research experience. Salary will be on the RS1A scale (£12,129 — £19,328) depending on qualifications and experience.

**Applications, comprising a C.V., statement of research interests, and the names of two referees, should be sent to Dr R E M Hedges, Research Laboratory for Archaeology, 6 Keble Road, Oxford OX1 3QJ, to whom preliminary enquiries about this post may be made (0865-273930). Closing date: 31st October.** *Oxford University is an Equal Opportunity Employer.* (9575)A

UNIVERSITY OF LIVERPOOL
Department of Biochemistry

## Postdoctoral Senior Research Assistant

Initial salary within the range £12,129-£14,359 per annum (under review).

Tenable for up to three years.

To study the structure and regulation of eukaryotic Ap4A hydrolases using novel nucleotide analogues. Applicants should have experience in one or more of the following: cDNA clonings, expression and related techniques, cell culture, and protein and synthetic chemistry.

Informal enquiries to Dr AG McLennan (051-794 4369) or Dr BT Barraclough (051-794 4327).

Applications, by c.v. with the names of three referees, should be received not later than 16 October 1992, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX,** from whom further particulars may be obtained.

Quote ref. RV/453/N. An Equal Opportunity Employer. (9549)A

## POSITIONS WANTED

**PhD-BIOCHEM/MOL BIOL: Skills exp mol cell biol & horml reg bone cells & transdtn-mechs. Sks ft pos immed challenging teaching, research, or combo. 10yrs in endo. Box NW8462B**

**PLANT PHYSIOLOGIST** 33, PhD, seeks challenging position, preferably in Europe or Australia. Six years post-doc experience in $N_2$-fixation research, stable isotope studies and ecophysiology; 20 publications. Speaks English and German. Box Number 9559D.

## BOX NUMBERS

To ensure your reply goes to the correct address please note the following: *Box Numbers carrying the code "NW" should be addressed as follows:* **Box Number NWxxx c/o Nature Classified, 65, Bleeker Street New York, NY 10012, U.S.A.** *All others please send to:* **Box Number xxxx, Nature Classified Production, 4, Essex Street, London WC2R 3LF, UK**

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| October 8th | Labweek (London, UK, Oct 13-15) | Copy deadline: Oct 2nd |
| October 15th | Electrophoresis Forum (Munich, FRG, Oct 26-28) | Copy deadline: Oct 9th |
| October 22nd | American Society for Neuroscience (Anaheim, USA, Oct 25-30) | Copy deadline: Oct 16th |
| October 29th | American Society for Human Genetics (San Francisco, USA, Nov 8-12) | Copy deadline: Oct 23rd |
| November 5th | Nature 15th International Conference (Boston, USA, Nov 12-13) | Copy deadline: Oct 30th |
| November 12th | American Society for Cell Biology (Denver, USA, Nov 15-19) Scientific Instruments Show (Tokyo, Japan, Nov 16-19) | Copy deadline: Nov 6th |
| November 19th | MAC (Milan, Italy, Nov 16-19) | Copy deadline: Nov 13 |

***For further details, and the latest copy deadlines, contact your nearest sales office NOW:***

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| Tokyo: | Tel: (03) 3 267 8751 | Fax: (03) 3 267 8746 |
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# Focus on Neuroscience

in conjunction with *Nature*'s attendance at the 5th Congress:
European College of Neuropsychopharmacology
(Marbella, Spain, 18-22 October 1992)

## Postdoctoral fellow in neurobiology

***Akzo, a leading multinational company, with headquarters in Arnhem - the Netherlands -, currently employes over 65.000 people within 350 companies operating in 50 countries. A position at the Akzo company means a career at a technologically high developed company with core activities in chemical products, man-made fibers, coatings and healthcare products.***

**Organon International bv**, one of the 6 companies within the Pharmaceutical Division of Akzo, is engaged in the development, production and marketing of drugs for human ethical use. More than 6,000 people are employed world-wide of whom roughly 1,000 are employed in the R&D organisation. Research is focused on fertility control, the nervous system, (cardio)vascular diseases, immunology and cancer.

**A postdoctoral position** (appointment for max 3 years) is available at the Department of Neuropharmacology at Oss, the Netherlands. The department is involved in the development of new psychotropic drugs and wants to expand the use of primary tissue cultures and (genetically modified) cell lines for the study of the mechanism of action of these drugs, including their postsynaptic intracellular and/or membrane responses.

For this position we are looking for a candidate with experience in neurochemical/histochemical and electrophysiological techniques as applied in these cell cultures. You should preferably have a background in pharmacology. At Organon you will find a scientifically stimulating environment and publication of research findings is highly encouraged.

AKZO
CREATING THE RIGHT CHEMISTRY.

**Information en application**
Further information can be obtained from dr. A. van Delft, head NPH department, telephone 31-412062073.
Applicants are asked to send in a statement of interest, curriculum vitae and a list of publications within 3 weeks to Organon International bv, attn. mrs. M. Bout, Personnel Department, PO Box 20, 5340 BH Oss, The Netherlands.

(W0146)A

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**University of Cambridge**
***Physiological Laboratory***
**SENSORY RECEPTORS**

Several postdoctoral positions are available, for up to 5 years, for the study of sensory transduction and adaptation in photoreceptors, olfactory receptors, hair cells and other mechanoreceptors. Applicants should preferably have a background in electrophysiology. Starting date is flexible.
**Send c.v. and names and addresses of up to 3 referees to Dr Trevor Lamb or Prof Andrew Crawford, Physiological Laboratory, Downing Street, Cambridge CB2 3EG, UK.**
**Fax: +44 223 333 840. E-mail: TDL1@Phx.Cam.AC.UK.**
**Funded by Wellcome Trust and E.C.**
***The University follows an equal opportunities policy.*** (9538)A

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***Assistant Professorship***
***in Visual Science***

The University of Rochester invites applications for a tenure-track position involving teaching and research. Available September 1, 1993, this position could reside in the departments of Psychology, Computer Science, Neurobiology and Anatomy, Ophthalmology, or Physiology. We are particularly interested in applicants with research interests in mechanisms of high level visual processing. Applicants must have a Ph.D., or an M.D with research experience.
Send by Jan. 1, 1993, curriculum vitae, research description and plans, reprints/preprints, and three names for recommendations to **David Williams, Center for Visual Science, University of Rochester, Rochester, NY 14627.** *The University of Rochester is an equal opportunity / affirmative action employer.* (NW8434)A

# Postdoctoral Molecular Biologist

DEPARTMENT OF PURE AND APPLIED BIOLOGY

Applications are invited for a three year appointment to join a molecular lab studying neurobiology and development in nematodes (*Caenorhabditis elegans* and parasitic nematodes). The work will involve the cloning and functional expression of G protein-coupled receptor and neurotransmitter transporter genes, and the generation of mutations in the *C.elegans* genes to probe function.

Applicants should hold or have submitted for a Ph.D. Experience of molecular techniques and familiarity with *in vitro* expression systems would be especially useful, but experience in *C.elegans* biology and parasitology is not essential.

Salary on the IA Range for Research Staff (£12,129 - £19,328) (pay award pending), according to qualifications and relevant experience.

Informal enquiries may be made to Dr R E Isaac (tel. 0532 332903) direct line.

Application forms and further particulars may be obtained from, and completed applications forwarded to, the **Academic Staff Office, The University of Leeds, Leeds LS2 9JT (tel. 0532 335771)** quoting reference no. 56/45. Closing date for applications: 29 October 1992.

The University of Leeds promotes an equal opportunities policy.

(9542)A



UNIVERSITY OF BRISTOL

## POSTDOCTORAL RESEARCH ASSISTANT

You will be working in the Departments of Physiology and Pharmacology with Dr S Lisney and Professor P Keen on a Wellcome Trust funded project concerning changes in neurogenic inflammatory responses triggered by peripheral nerve injury. The post is for 1½ years in the first instance and is available immediately. Some experience of peripheral nerve electrophysiology and/or measurement of neuropeptides by radiommunoassay would be an advantage but is not essential.

Applications should be sent to Dr Lisney, from whom further information can be obtained, at the Department of Physiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD. Tel: (0272) 303461. Closing date: 22nd October 1992.

An Equal Opportunities Employer. (9556)A

## Postdoctoral Electrophysiologist

***Department of Pharmacology***
***Bristol University UK***

A postdoctoral position is available, following the award of a programme grant by the MRC, to study the role of neuronal calcium channels in adaptive mechanisms. Experience in electrophysiological techniques (preferably patch-clamp or voltage-clamp) is required. Appointment will be for up to five years in the first instance.

**Contact Dr. H. J. Little, Pharmacology Department, The Medical School, University Walk, Bristol BS8 1TD, with CV and names of two referees.** (9530)

**UNIVERSITY OF CAMBRIDGE**
**DEPARTMENT OF PHARMACOLOGY**

## *Rhône-Poulenc Rorer Post-Doctoral Research Fellow*

Applications are invited for a position of Research Fellow to work in the Drug Design Group on the development of novel computer algorithms for the incorporation of hydrophobicity into site-directed drug design. The applicant should have experience in drug design strategies for known crystallographically defined sites and an interest in the energetic and entropic effects introduced by the generation of novel ligands within their binding sites.

The appointment is for 3 years with an age-related salary in the range £12,129 – £19,328. Applications, with C.V. and names of two referees, should be sent before 19th October to **Dr. P. M. Dean, Department of Pharmacology, Tennis Court Road, Cambridge CB2 1QJ.** (9531)A

**UNIVERSITY OF LIVERPOOL**
**Department of Physiology**

## Lecturer

Initial salary within the range £12,860-£23,739 per annum (under review).

Tenable from 1 April 1992.

Vacant following the appointment of John Garthwaite as Director of Neuroscience Research at the Wellcome Foundation. Applicants should have made substantial research contributions and be able to establish a vigorous and independent research group in our new Physiology Research Building. The established main research groups are working on: Molecular mechanisms of exocytosis (Professor Burgoyne), Regulatory peptides in the gastrointestinal tract (Professor Dockray, Drs Dimaline and Varro), $Ca^{2+}$ signals and stimulus-secretion coupling (Professor Petersen, Mr Gallacher and Dr Tepikin) and Regulation of smooth muscle contraction in the uterus (Dr Wray). The department teaches a wide range of courses in the faculties of Medicine and Sciences and the appointee would participate in several of these. The successful applicant would be expected to take up this post in the Spring, 1993.

Informal enquiries to Professor Burgoyne (051-794 5305), Professor Dockray (051-794 5325) or the Head of the Department, Professor Petersen (051-794 5322).

Applications by c.v. with the names of three referees, should be received not later than 5 November 1992, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX,** from whom further particulars may be obtained.

Quote ref. RV/454/N. An Equal Opportunity Employer. (9548)A

# Postdoctoral fellowship Computational Chemistry

*Akzo, a leading multinational company, with headquarters in Arnhem - the Netherlands -, currently employes over 65.000 people within 350 companies operating in 50 countries. A position at the Akzo company means a career at a technologically high developed company with core activities in chemical products, man-made fibers, coatings and healthcare products.*

**Organon International bv**, - part of the Pharma Division of Akzo - is engaged in the development, production and marketing of drugs for human ethical use. More than 6,000 people are employed world-wide of whom roughly 1,000 are employed in the R&D organisation. Research is focused on fertility control, immunology, (cardio) vascular diseases, the nervous system and cancer.

**A postdoctoral position** (appointment for a maximum of 3 years) is available at the Department of Computational Medicinal Chemistry (CMC) at Oss, the Netherlands. The department aims to support the rational design of biologically active compounds with a pre-defined pharmacological profile. In this respect, computational chemistry techniques (molecular modelling and graphics, energy calculations,QSAR) and database techniques are employed.

The department is involved in the development of 3D databases and 3D search techniques (e.g. *Chemical & Engineering News*, August 10, 1992, p 20).

Your job will mainly involve the in-house development of CMC methodology. The work will be focused on *de-novo* drug design approaches and partly be a continuation of 3D search strategies. We expect you to be capable of carrying out a research project in computational chemistry and to have demonstrated this ability by obtaining a PhD degree in chemistry with emphasis on computer applications. At Organon you will find a scientifically stimulating environment and many possibilities for further personal development. We expect you to communicate results at meetings as well as through scientific publications. Salary will be commensurate with experience with a minimum of NLG 5,028 per month.

AKZO
CREATING THE RIGHT CHEMISTRY.

**Information en application**
Further information can be obtained from dr. J.R. Mellema, head CMC department, telephone +31-4120-62140.
Applicants are asked to send in a statement of interest, resumé (CV) and a list of publications within 3 weeks from the date of issue to Organon International bv, attn. J.J.M. Hoeven, Personnel Affairs, PO Box 20, 5340 BH Oss, The Netherlands (or faxed to +31-4120-62539).

(W0147)E

**UNIVERSITY OF SOUTHAMPTON**
SCHOOL OF BIOLOGICAL SCIENCES
DEPARTMENT OF BIOLOGY

## POST-DOCTORAL RESEARCH ASSISTANT IN DEVELOPMENTAL NEUROBIOLOGY

A post-doctoral research assistant is required to work on an SERC funded project on the growth and differentiation of identified neurons in insect embryos.

Experience in the field of neurobiology or animal development would be an advantage but is not essential.

The appointment is for three years to commence as soon as possible and will be on Research Grade 1A

Informal enquiries may be made to Dr D Shepherd on (0703) 594389.

Applications (2 copies of curriculum vitae, the names, addresses and telephone numbers of 2 referees and 2 copies of all correspondence relating to your application) should be sent to the Personnel Department (S), University of Southampton, Highfield, Southampton SO9 5NH to arrive no later than 31 October 1992. Please quote reference S/52/NA. (9541)A

UNIVERSITY OF BRISTOL

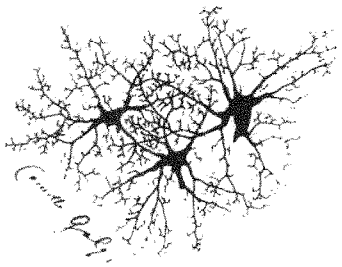
## TEMPORARY LECTURESHIP

Applications are invited for a Temporary Lectureship in the **Department of Physiology** for 2 years from January 1993 or as soon after as possible. Candidates should be prepared to lecture, mainly to Medical Students, in Cell Biology, Histology and some areas of Physiology and to run practical classes in Histology. The successful candidate would be encouraged to collaborate in research with Dr SN Lawson's or another suitable research group in the department.

Salary: £12,860 - £17,827 (under review) on the Lecturer Grade A scale.

For further details telephone Bristol (0272) 256450 (ansaphone after 5pm) or write to the Personnel Office (EO), University of Bristol, Senate House, Bristol, BS8 1TH, to whom applications including curriculum vitae and names of two academic referees, should be sent by 23rd October 1992. Please quote reference A338R

An Equal Opportunities Employer. (9557)A



# 2ND "C. GOLGI" WINTER CONFERENCE ON NEUROSCIENCE

**"Molecular pathophysiology of brain diseases: Alzheimer's disease and related neurodegenerative disorders"**

**Ponte di Legno, Italy,**

**January 26 – 29, 1993**

**Organizers:** F. Cattabeni (Italy), W. H. Gispen (The Netherlands), P. F. Spano (Italy).

**Topics:**

Structural pathology of the cytoskeleton.
Beta-amyloid: expression, processing and neurotoxicity.
Calcium homeostasis and neurodegeneration.
Alzheimer's disease: from experimental to human studies.

**Provisional list of speakers:**

G. Bellomo (Italy), K. Beyreuther (Germany), J. P. Brion (Belgium), O. Bugiani (Italy), J. D. Buxbaum (USA), C. Cotman (USA), A. Delacourte (France), J. Disterhoft (USA), D. Perani (Italy), W.H. Gispen (The Netherlands), A. Goate (England), M. G. Mattson (USA), R. Nitsch (USA), M. Roth (England), T. Schuurman (Germany), M. Trabucchi (Italy).

The number of participants is limited to 70.

*Application deadline: December 15th, 1992.*

Address correspondence to:

**Organizing Secretariat**
**Brita Rolander Chilo**
**Nutrition Foundation of Italy**
**Via S. Pietro all'Orto, 17**
**Milano — Italy**

**Tel/Fax (39)(2) 76003514.**

(W0160)C

UNIVERSITY OF NEWCASTLE UPON TYNE
MUSCULAR DYSTROPHY GROUP RESEARCH LABORATORIES

## RESEARCH ASSOCIATE

Applications are invited for a postdoctoral vacancy to study the function, distribution and developmental regulation of voltage-dependent sodium channels at the mammalian neuromuscular junction. The work will be carried out in a group recognised internationally for its work on the structure and function of the neuromuscular junction in animals and man. A variety of techniques including intracellular recording and voltage clamp, ligand binding, quantitative video flourescence microscopy and electron microscopy will be used to study the involvement of voltage-dependent sodium channels in the generation of muscle fibre action potentials. There will also be an opportunity to take part in ongoing studies of neuromuscular junctions in patients with a variety of neuromuscular disorders. While a wide variety of backgrounds might be suitable for this project including physiology, biophysics, biochemistry, cell biology etc., some experience in electrophysiology would be desirable.

The post is available immediately and will hopefully be taken up by 1 January 1993, and is tenable for three years. Salary will be at an appropriate point on the 1A salary scale £12,129 – £19,328 per annum depending upon qualifications and experience.

Three copies of applications, including curriculum vitae and the names of three referees should be forwarded to **Dr C R Slater, Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE (Tel: 091-273 8811, ext. 22180)** from whom further particulars are available on request. Closing date for applications is 24 October 1992.

Please quote ref. 0814/N. (9565)A

## FACULTY POSITION
## CELL/MOLECULAR BIOLOGIST
## Department of Physiology
## McGill University

Applications are invited for a tenure track position at the assistant professor level. The successful applicant will be expected to initiate an independent research program emphasizing molecular biological techniques. Research areas of special interests include ion channels, receptor-mediated signal transductions, cytokines/growth factors, cell adhesion and molecular biology of the lung. Applicants should have a doctoral degree, significant postdoctoral research experience and a demonstrated record of research accomplishments. Send curriculum vitae, bibliography, statement of research interests and future plans and the names of three references by **Jan. 15, 1993 to: Dr. David Goltzman, Chairman, Department of Physiology, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6.** *While all eligible candidates are encouraged to apply, initial preference will be given to applicants who are Canadian citizens or permanent residents of Canada in accordance with Canadian Immigration regulations.* (NW8475)A

# The Wellcome Trust

# Postdoctoral Scientists
# Final Year PhD Students / Final Year Undergraduate Students

The Trust wishes to promote research in **molecular** and **cellular** aspects of **toxicology** which for this purpose is defined as 'the study of how chemical and physical agents cause deleterious effects on living systems'. Therefore, in addition to funding project grants, the Trust will support individuals wishing to develop careers in this area in two ways.

## Postdoctoral Research Fellowships

**Tenable** for up to three years in an appropriate academic institution in the UK or Republic of Ireland.

**Eligibility:** candidates must have been engaged in research in the UK or Republic of Ireland for the last three years. Candidates who have experience in a relevant scientific discipline and now wish to develop a career in toxicology are particularly encouraged to apply: such applications should include details of the training to be provided during the fellowship. Proposals from postdoctoral toxicologists who wish to develop an independent research programme are also welcomed. **The salary** will be based on the Trust's fellowship scales which are aligned to the national academic scales.

## Research Studentships

**Tenable** for three years in an appropriate academic institution in the UK or Republic of Ireland.

**Eligibility:** applicants should have, or expect to attain, at least a Class II(i) degree in an appropriate subject.

**Stipend and research expenses** will be at the advantageous level offered to holders of Wellcome Prize Studentships.

Prospective applicants for either scheme are welcome to discuss their proposals with the scientific staff of the Trust. Requests for application forms should be sent to:

**Until 17th October 1992**: The Grants Section (T/F), The Wellcome Trust, One Park Square West, London NW1 4LJ.
Tel: 071-486 4902. Fax: 071-487 4915.

**After 17th October 1992**: The Grants Section (T/F), The Wellcome Trust, 183 Euston Road, London NW1 2BE.
Tel: 071-611 8888. Fax: 071-611 8545.

All requests should include a letter of support from the Head of Department in which the candidate wishes to work, a brief CV and an outline (500 words) of the proposed research.

Those short-listed for Research Fellowships will be invited to the Trust Offices for interview. Candidates applying for Toxicology Studentships are not eligible for consideration under the general Wellcome Prize Studentship scheme.

The closing date for applications is 1st February 1993. Late applications will not be accepted.

(9546)E/F

# HUBBLE POSTDOCTORAL FELLOWSHIPS

The Space Telescope Science Institute announces the continuation of the Hubble Fellowship Program in cooperation with astronomical institutions throughout the United States. The main objective of the Program is to provide recent postdoctoral scientists of unusual promise and ability opportunities for research on problems, largely of their own choosing, that are related to the Hubble Space Telescope science and compatible with the interests of the host institutions. The program is open to scientists of any nationality who have earned their doctorates after January 1, 1990, in astronomy, physics, or related disciplines.

The duration of the fellowship is for a total of three years, which includes an initial appointment of two years and an extension to a third year, contingent on a positive mid-term review. Contingent on funding from NASA, up to 15 new Hubble Fellows will be supported this year by grants to United States institutions.

The Announcement of Opportunity, including the application instruction, may be obtained from the address listed below. The application deadline is November 20, 1992. The new Hubble Fellow appointments are expected to begin on September 1, 1993. Minorities and women are strongly encouraged to apply. EEO/AA/M/F/D/V.

**Hubble Postdoctoral Fellowships**
**SPACE TELESCOPE SCIENCE INSTITUTE**
**3700 San Martin Drive • Baltimore, MD 21218**
**SPAN:6559::HFELLOWS**
**BITNET: HFELLOWS@STSCI.BITNET**
**INTERNET: HFELLOWS@STSCI.EDU**
**Attention: Hubble Fellowship Program Office**

SPACE TELESCOPE SCIENCE INSTITUTE (NW8459)E

# Post Doctoral Fellowship
## In Plant Molecular Biology

**3 Year Term**

**$36K-$39K Salary + Superannuation**

*Division of Plant Industry, Canberra ACT, Australia*

The aim of this project is to improve the malting quality of barley by genetic engineering. We are seeking a skilled and experienced molecular biologist to design and construct improved genes coding for enzymes which play key roles in the malting process. Initially, this will involve barley a-amylase and barley b-glucanase but other genes will be incorporated into the program as they become available. The improved genes will be used to transform barley and you will be involved in the assessment of transgenic plants for improved malting quality. This work will be done within a research program which seeks to understand the molecular processes occurring in the endosperm of germinating barley grain. Previous experience with recombinant DNA procedures is essential.

Dr Jake Jacobsen, telephone (61 6) 246 5464, fax (61 6) 246 5000 or Dr Geoff Fincher, (La Trobe University, Bundoora, Victoria), telephone (61 3) 479 2158 or fax (61 3) 479 2467, can provide you with more information about the job. Duty statement and selection criteria can be obtained from Lynette Webb, telephone (61 6) 246 5295 or fax (61 6) 246 5000.

Please send your application for the position above, quoting Ref. No. PG92036NAT and include details of your experience, skills, and qualifications, marked "confidential" to: **The Recruitment Office, CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia** by October 30, 1992.

(W0149)E

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

CSIRO AUSTRALIA

94619

# AFRC–INRA FELLOWSHIPS 1993-94

Applications are invited from scientists working in AFRC Institutes and British higher education institutions for AFRC/INRA Fellowships tenable during the period of January 1993 to January 1994.

These fellowships are intended to provide a further stimulus to the development of collaboration between INRA and British scientists working in the biological and other sciences supporting agriculture, biotechnology and food production.

Priority areas include environmental research, development biology, animal welfare, genome analysis and food sciences.

They are tenable in any INRA Institute for a period of up to twelve months, with a maximum value of £800 per month to cover travel and personal subsistence costs.

For an application form and other details, contact Neville Ashcroft, International Branch, AFRC Central Office, Polaris House, North Star Avenue, Swindon SN2 1UH. (Tel:- 0793 413027, Fax:- 0793 413201).

Closing date for applications is 30 October 1992. Applicants will be notified by 30 November 1992. (9527)E

*Agricultural & Food Research Council*

AFRC INRA

# EMBO

## Additional Postdoctoral Fellowships for East European Molecular Biologists

The EMBO, as the result of a second special voluntary contribution to the budget of the EMBC from the Government of the Federal Republic of Germany, consequent upon the reunification of Germany, is able to make available additional 15 fellowships to allow molecular biologists from East European countries to work in laboratories in the Member States of the EMBC.

The deadline for applications for these fellowships is 1 November 1992, and successful candidates will be notified by 15 December 1992.

The conditions applying to these special fellowships are as follows:

1. applicants must be residents of an East European country and be working in that country at the time of application;
2. applicants must hold a PhD degree and be under the age of 40 at the beginning of the fellowship;
3. applicants must provide a well defined research proposal to be carried out in a specific host laboratory in Western Europe or Israel;
4. the fellowships will be awarded for a period of between three months and a maximum of six months, and they will not be renewable or extended beyond six months;
5. the fellowships will provide a subsistence allowance and a travel grant but they will not include any financial support for dependants.

Application forms can be obtained from: The Executive Secretary, EMBO, Postfach 1022.40, DW-6900 Heidelberg, Germany; Telephone 49-6221-383031; telefax 49-6221-384879. (W0006)E

## University College London
## RAMSAY MEMORIAL FELLOWSHIP FOR CHEMICAL RESEARCH

UCL

The Trustees will consider in January 1993 applications for the award of one or more Ramsay Fellowships, which may be co-sponsored by a British industrial concern. The Fellowships will normally be tenable in the United Kingdom for two years from 1 October 1993. Candidates will generally be expected to show evidence of research experience since obtaining a PhD, although it is desirable that post-doctoral work should normally not have exceeded two years, and the age on taking up appointment should not be more than 28 years. The maximum age limit for applicants is 35. The value of the Fellowship is usually comparable with the lower part of the Lecturer's salary scale for British universities, to which may be added a grant for expenses not exceeding £100 per annum. In addition, USS or similar superannuation arrangements may be made. Particulars from the Ramsay Memorial Fellowship Trust, University College London, Gower Street, London WC1E 6BT. Completed application forms must be received by 15 November 1992. An Equal Opportunities Employer. (9536)E

# ROCKEFELLER FOUNDATION BIOTECHNOLOGY CAREER FELLOWSHIPS

The Rockefeller Foundation announces a program of career development fellowships designed to enable scientists from developing countries, trained at outstanding centers for advanced research on biotechnology, to continue to work at those or other institutions for three months each year, over a period of at least three years, conducting advanced research and keeping abreast of new developments in their fields. The program will focus upon the development and application of advances in molecular and cellular biology and immunology relevant to agriculture, health, reproductive biology and environmental protection. Funding will be shared between the Foundation and the host laboratory, with the Foundation providing travel and per diem support. It is hoped that the fellowships will encourage the establishment of ongoing working relationships between outstanding younger scientists working at third world institutions, and research teams at advanced laboratories.

Applicants to this program should have at least Ph.D.-or M.D.-level training, a proven record of scientific productivity, and a permanent position at a research or teaching institution in their home country. A written project proposal must be developed and submitted jointly by the candidate and the laboratory sponsor.

Information about application procedures can be obtained by writing to **Biotechnology Career Fellowships, Fellowship Office, Rockefeller Foundation, 1133 Avenue of the Americans, New York, New York 10036, USA.** (NW7728)E

# *Alternative Thinking Fellowships*

The Alternative Thinking Program has been designed to support research and scholarship which represent new departures within and across disciplines. It is intended to encourage the development and expression of minority views which challenge received doctrines and paradigms, and in this way to promote rethinking across all fields of knowledge. The program offers full research fellowships (one or two semesters, extendible). Send applications (including C.V. & research proposals) to Prof. Meir Sternberg, Faculty of Humanities, Tel Aviv University, Tel Aviv, Israel. (W0029)E

## WYE COLLEGE — UNIVERSITY OF LONDON

**Department of Biochemistry and Biological Sciences in collaboration with Horticulture Research International**

## AFRC POSTDOCTORAL RESEARCH FELLOWSHIP AND GRADUATE TECHNICIAN IN MOLECULAR PLANT PATHOLOGY

Applications are invited for a Postdoctoral Research Fellowship and a Graduate Technician in molecular plant pathology. The posts are to undertake work on the mapping and cloning of genes from *Arabidopsis thaliana* that code for resistance to the fungal pathogen *Peronospora parasitica*. The positions are funded for four years (on the Research Assistant 1A Scale from £12,129 per annum and the Graduate Technician Grade D from £11,018, according to age and experience) under the AFRC Plant Molecular Biology Programme. The work will invole RFLP and RAPD mapping, YAC cloning and the analysis of gene function. The positions are to join an ongoing coordinated research program between HRI East Malling and Wye College aimed at understanding and exploiting gene-for-gene interactions in plants.

Applications should be sent to **Dr. T. A. Hill, Assistant Director of Administration, Wye College, University of London, Ashford, Kent TN25 5AH, England, Telephone 0233 812401, ext. 228, Fax 0233 813320** from whom further particulars are available. Applications should include a covering letter, curriculum vitae, list of publications and the names of three individuals from whom references may be sought. Applicants wishing to discuss the post further are invited to contact Dr Jim Beynon on the above telephone number, Extension 483 or Fax 0233 813140.

*Wye College is an Equal Opportunities Employer.* (9572)E

## WYE COLLEGE
## UNIVERSITY OF LONDON

**The Plant Molecular Biology Laboratory**
**Department of Biochemistry and Biological Sciences**

***SERC Postdoctoral Research Fellowship in Molecular Biology of Plant Development***

Applications are invited for a Postdoctoral Research Fellowship in plant molecular biology. The post is to undertake work aimed at the analysis of the regulation of genes that are induced during leaf senescence in *Brassica napus*. The work will involve the isolation and characterisation of the promoters of several senescence specific genes that have been identified in this laboratory. This analysis should help to identify the regulatory elements that are involved in this key step in plant development. The position is funded for three years (on the Research Assistant 1A scale: £12,129 to £19,328 per annum according to age and experience).

Applications should be sent to **Dr. T. A. Hill, Assistant Director of Administration, Wye College, University of London, Ashford, Kent TN25 5AH, England. Telephone 0233 812401, ext. 228, Fax 0233 813320** from whom further particulars are available. Applications should include a covering letter, curriculum vitae, list of publications and the names of three individuals from whom references may be sought. Applicants wishing to discuss the post further are invited to contact Dr. Vicky Buchanan-Wollaston on the above telephone number, Extension 375 or Fax 0233 813140.

*Wye College is an Equal Opportunities Employer.* (9573)E

## FELLOWSHIPS & STUDENTSHIPS IN OCEANOGRAPHY

Fellowships & (MSc, PhD, and Post-Doctoral) are available at Delhousie University, Department of Oceanography, for applicants with a strong preparation in physics (radiative transfer, atmospheric or marine optics, mathematical modelling), or biology (algal physiology, photosynthesis). Research in progress includes applications of remotely-sensed data on ocean colour: scattering of light in the sea; photosynthetic pigments and light absorption; parametrisation of photosynthesis in the sea: coupling of biological models to ocean circulation models. Research in related fields could be acceptable. **Please contact Dr. Trevor Platt or Dr Shubha Sathyendranath, Bedford Institute of Oceangraphy, Dartmouth, Nova Scotia, Canada B2Y 4A2. Phone (902) 426-3793: Fax (902) 426 9388.** (NW8463)E/F



## OBSERVATOIRE DE LA CÔTE D'AZUR

## Henri Poincaré POSTDOCTORAL FELLOWSHIPS

partially supported by

**Conseil Général des Alpes-Maritimes**

Astronomy, interferometry, nonlinear dynamics and massively parallel scientific computations are among the areas of active research at OCA for which postdoctoral applications are invited. The fellowships are for one year (exceptionally renewable once). There are two positions per annum. Fellowships range from 10 000 FF to 15 000 FF per month, depending on experience. Applicants should have obtained a Ph.D. outside of France within the last ten years. Applications will be reviewed internally, with the final selection done by the Comité Henri Poincaré, comprising representatives of Conseil Général des Alpes-Maritimes, Académie des Sciences, Collège de France and funding agencies. Positions will start on September 1, 1993. Informal enquiries: Mrs C. Caseneuve, phone: +33 92 00 30 46, bitnet: cpoca@froni51, span: 17499::cpoca. Closing date: 15th January 1993.

Four copies of applications, including CV, description of research and names of three referees and one set of key publications should be sent with address, telephone, e-mail and fax of applicant and suggested referees to: *Secrétariat du Comité Postdoctoral de l'Observatoire de la Côte d'Azur, Observatoire de Nice, B.P. 229, 06304 — Nice Cedex 4, France. (Fax +33 92 00 30 58).*

*The Observatoire de la Côte d'Azur (OCA) is located near the cities of Nice and Grasse in southeastern France. OCA is supported by the Ministry of Education Nationale and is associated to Centre National de la Recherche Scientifique. Additional supporting agencies include the European Community.* (W0148)E

PROGRAMME

## NERC INITIATIVE in Taxonomy Research and Training

NERC is providing c.£2m for a 5 year programme to help reinvigorate taxonomy research and training within UK higher education institutions (HEIs).

Packages of support will be provided to three HEIs, each comprising:

- NERC Fellowships in Taxonomy
- Research grants
- Support for postgraduate students to attend established short courses.

Applications from HEIs (not from individuals) are invited by 1st December 1992.

Information is available from Miss Mary Mochrie, NERC, Polaris House, Swindon SN2 1EU. Telephone (0793) 411758. Those who have already requested details will be sent information directly. (9564)O

Natural Environment Research Council

# Public Announcement Regarding a New Research and Development Project on Ultimate Manipulation of Atoms and Molecules

*Announced by the New Energy and Industrial Technology Development Organization on October 1, 1992*

*In order to promote the research and development of industrial technology, the New Energy and Industrial Technology Development Organization (NEDO) would like to inform all interested companies and research organizations regarding the research and development project described below. This new project is being undertaken as part of the National Research and Development Program (the Large-Scale Project) of the Agency of Industrial Science and Technology, Ministry of International Trade and Industry of Japan.*

**Theme of the Research and Development Project**
"Ultimate Manipulation of Atoms and Molecules".

**Outline of the Research and Development Work to be Entrusted**
The purpose of this project is to develop techniques for probing and manipulating atoms and molecules on solid surfaces or in three-dimensional space with extreme precision, and their support techniques, as common fundamental technology for such fields as new materials, electronics and biotechnology.

**Style of the Research and Development**
This project will be implemented as joint research and development between a national laboratory and project participants.

The project's research activities will be carried out at one place especially organized for this project.

**Procedures for Application**
*(1) Qualification Criteria*
All companies or research organizations who meet the following qualification criteria may submit an application to participate in the above project:

1. The applicant must be a company or organization having human resources who are experienced and well qualified to carry out the research and development in the field covered by or related to the project.
2. The applicant must be in sound financial condition and have the ability to manage its finances as necessary to smoothly carry out the project work.
3. The applicant must be able to comply with NEDO's instructions, if such are necessary, to fully carry out the project work.
4. The applicant must have attended the explanatory meeting held by NEDO as set forth in item (2) below or been represented at the meeting by a responsible agent or representative who is capable of accurately conveying the contents of the meeting in detail.

*(2) Explanatory Meeting*
An explanatory meeting will be held on the date shown below in order for NEDO to fully explain the details of the project's research and development work to be entrusted and the application documents to be submitted. All companies or research organizations who are interested in submitting an application to participate in the project are required to attend this meeting or to send an agent or representative to attend on their behalf. Japanese will be the only language used during the meeting.

**Date:** *Friday, October 16, 1992*
**Time:** *14:00 to 15:00*
**Place:** *NEDO's Head Office*
*30th Floor, Sunshine 60 Building,*
*1-1, Higashi-Ikebukuro 3-Chome*
*Toshima-ku, Tokyo 170*

*(3) Further Information*
For further information regarding the research and development work to be entrusted under the above project, please contact NEDO by telefax as follows:

**New Energy and Industrial Technology Development Organization**
**Contract Division, Accounting Department**
**28th Floor, Sunshine 60 Building**
**1-1, Higashi-Ikebukuro 3-Chome**
**Toshima-ku, Tokyo 170 Japan**
**Telefax: 08-5992-1184**

## COURSES

A School of the University of London

**Royal Postgraduate Medical School**

# Immunolabelling for Electron Microscopy

***18th-20th January 1993***

**A three day intensive practical course which will cover:**

*Preparation and processing of tissue*
*Colloidal gold techniques*
*Cryoultramicrotomy*
*Multiple staining procedures*

**Course organisers:** Dr C Sarraf (Histopathology)
Professor J M Polak (Histochemistry)

**Course fee (inc catering):** **£250.00**

**Further details and application forms from:** Wolfson Conference Centre
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 0NN

**Telephone:** 081 740 3117/3245

**Fax:** 081 740 4950

*This course now receives full US-CME accreditation*

(9568)D

**STRUCTURE, BIOGENESIS AND DYNAMICS OF BIOLOGICAL MEMBRANES**

NATO ADVANCED STUDY INSTITUTE CO-SPONSORED BY FEBS
JUNE 14 — 26 - 1993 - CARGESE - CORSICA - FRANCE

**Lipid Dynamics & Lipid-Protein Interactions:** B. de Kruijff (Utrecht), W. Dowhan (Houston), W.J. Lennarz (Stony Brook), J.A.F. Opden Kamp (Utrecht), J. Seelig (Basel), A. Watts (Oxford).

**Protein Translocation & Insertion:** W. Neupert (München), R. Schekman (Berkeley), G. Von Heijne (Huddinge), P. Walter (San Francisco), W. Wickner (Los Angeles).

**Intracellular Traffic:** V. Bankaitis (Urbana), J. Gruenberg (Heidelberg), S.R. Pfeffer (Stanford), G. Warren (London).

**Protein Structure & Folding:** T.E. Creighton (Heidelberg), L.M. Gierasch (Dallas), C. Lazdunski (Marseille), G.E. Schulz (Freiburg).

The A.S.I. will feature lectures, poster sessions and (in)formal discussions and will be open for graduate students, postdocs as well as specialists in this field.

Application forms and further information available from: **J.A.F. Op den Kamp, Centre for Biomembranes and Lipid Enzymology, University of Utrecht, P.O. Box 80.054, 3508 TB Utrecht, The Netherlands.** (W0151)D

Please mention
**nature**
when replying to these advertisements

## SCHOLARSHIPS

# ISREC Graduate Student Scholarships

ISREC

The Swiss Institute for Experimental Cancer Research (ISREC) invites applications for a competitive 3-year scholarship leading to completion of a PhD.

Eligible are students that have or are about to obtain an undergraduate degree from a European university. Successful candidates will choose in which ISREC research group they will carry out their thesis work: Chromatin (S. Gasser), Nucleus (E. Nigg), Transcriptional elongation (J. Mirkovitch), Cell cycle (V. Simanis), Post-transcriptional regulation (L. Kühn), Cellular differentiation (O. Hagenbüchle, P. Wellauer), T lymphocyte biology (M. Nabholz), Oncogenes (R. Iggo), Carcinogenesis (P. Cerutti, P. Amstad), Mutagenesis (R. Tyrrell), Virology of small DNA viruses (P. Beard, B. Hirt), Biology of membranes (J.-P. Kraehenbuhl), Experimental pathology (B. Sordat), Growth regulation of tumor cells (N. Odartchenko), Immuno- diagnostics and therapy of cancer (J.-P. Mach).

**Applications consisting of a curriculum vitae and the names of two academic referees should be sent by Nov 15th, 1992, to: Markus Nabholz, ISREC, 155, chemin des Boveresses, CH-1066 Epalinges s/Lausanne, Switzerland.** (W0152)I

## LECTURE

# LEUKAEMIA RESEARCH FUND

**1992 Guest Lecture**

will be delivered by

**Professor Laurent Degos**

**'Differentiation Therapy & Leukaemia'**

on Wednesday, 11th November, at 5.30 p.m.
at Regent's College, Regent's Park, London NW1
Tickets issued free of charge from:

**Leukaemia Research Fund,**
43 Great Ormond Street,
London WC1N 3JJ. Tel: 071-405 0101

(9551)K

## AWARDS

**MEDICAL RESEARCH SOCIETY**

# YOUNG INVESTIGATOR AWARDS

The MRS has instituted a Young Investigator session at its meetings in April. These provide a forum for research fellows completing their projects to review their work for each other and invited members of national Training Fellowship Award Panels. Submissions for the sessions will be selected for oral and poster presentations, and be eligible for the MRS medal and award of £500, kindly donated by Glaxo. The second session will be held at the MRS meeting, University College Hospital, London, on April 22nd 1993. Submissions should consist of a summary of the completed research on two sides of A4, together with a list of resulting publications. The sessions are open to all biomedical research fellows (clinical and non-clinical), who have undertaken a period of supervised research in the UK intended to lead to a doctoral degree. Applicants should be 35 or under on 31st December, 1993. Participants will be invited to become members of the MRS.

**Submissions should reach Dr JM Rhodes, MRS Secretary, Royal Liverpool University Hospital, Link 5Z, Prescot Street, Liverpool L7 8XP by December, 23rd 1992, enclosing a stamped addressed card if applicants wish acknowledgement.**

(9566)K

# THE SECOND 21st-CENTURY EARTH AWARDS

## Global Environmental Problem Proposal Competition
## Proposal-Toward a Lasting Earth

**ORGANIZERS** : Nihon Keizai Shimbun, Inc./Global Industrial and Social Progress Research Institute (GISPRI)
**SUPPORTERS** (tentative) : Ministry of International Trade and Industry/Environment Agency/Ministry of Foreign Affairs/Ministry of Education
**COOPERATORS** : The United Nations University/Japan Junior Chamber Inc./International Institute for Applied Systems Analysis/Resources for the Future
TATA Energy Research Institute/The Royal Institute of International Affairs/World Resources Institute
**SPONSOR** : Toho Mutual Life Insurance Co.

### OVERALL THEME

**Proposal-Toward a Lasting Earth**

### ENTRY CONTENTS

**GENERAL STUDY SECTION** : Researched proposals for the amelioration or solution of such global environmental problems as climate charge, deforestation or biodiversity preservation.
**LIFESTYLE PROPOSAL SECTION** : Environment-related proposals with immediate relevance to everyday life, indicating concrete suggestions for behavior policies for the general consumer.

### ENTRY QUALIFICATIONS

Individual(s).Entries for the Lifestyle Proposal Section can be accepted only from residents of Japan.

### METHOD OF ENTRY

Articles must be written by Japanese or English,The following details should be provided on the prescribed application from or on the cover of the submitted article Address,Name of author or group representative, Age, Sex, Profession, Affiliation or school, Submitted entries will not be returned.
**GENERAL STUDY SECTION** : Papers should be 20 to 40 pages (400 characters per page) in Japanese or 15 to 30 typed (double-spaced A4) pages in English. They should be accompanied by an abstract of up to 600 characters (250 words).
Only unpublished original papers will be accepted. Entries based on the contents of materials recentry presented in such forms as academic journals, journals of professional associations and academic symposia will, however, also be accepted. (In such cases, the source of the materials included should be specified.)
**LIFESTYLE PROPOSAL SECTION** : Works should be up to 15 pages (400 characters per page) in Japanese.Only unpublished orginal papers will be accepted.
* The specified length of articles in both sections includes the accompanying materials and bibliography.
**DEADLINE** : December 25 1992.

### CRITERIA FOR AWARDS

The following criteria shall be applied in the judging of entries for both sections :
1. Originality of the proposal. 2. Logical consistency of the proposal. 3. Freedom from need for experimental confirmation or verification. 4. Comprehensibility of proposal for general readers and accessibility for intelligent evaluation by said readers. 5.Feasibility of the proposal.

### SCREENING COMMITTEE MEMBERS

Takashi Mukaibo (Chairman, Global Indutrial and Social Progress Policy Forum/Professor Emeritus, The University of Tokyo) Hisashi Ishitani (Professor,The University of Tokyo) Yoichiro Ichioka (Director Chief Editorial Writer,Nihon Keizai Shimbun, Inc.) Yoichi Kaya (Professor, The University of Tokyo) Jiro Kondo (President,Science Council of Japan) Katsuo Seiki (Executive Director, Global Indusrial and Social Progress Research Institute 〈GISPRI〉 Kei Takeuchi (Professor, The University of Tokyo) Keiko Nakamura (Professor, Waseda University) Katsuya Nagata (Professor, Waseda University) Shinji Fukukawa (Advisor to Global Industrial and Social Progress Research Institute 〈GISPRI〉/ Former Vice Minister, Ministry of International Trade and Industry)
Evaluation committee(in random order with honorifics omitted) : Committee members from MITI and the Environment Agency are also expected to participate.

### AWARDS

All prizes are subject to government taxes.
**GENERAL STUDY SECTION** : 21st-Century Earth Award (one cash prize of 5 million yen) ; Nihon Keizai Shimbun Award (one cash prize of 2 million yen) ; GISPRI Award (one cash prize of 2 million yen)
**LIFESTYLE PROPOSAL SECTION** : 21st-Century Earth Award (one cash prize of 1 million yen) ; Nihon Keizai Shimbun Award (one cash prize of 300,000 yen) ; GISPRI Award (one cash prize of 300,000 yen) ; also commendation awards given as appropriate.

### COPYRIGHTS FOR AWARD-WINNING ENTRIES

The copyrights for the winners of the 21st-Century Earth Award, the Nihon Keizai Shimbun Award and the GISPRI Award shall belong to the organizers, Publishing and merchandising of award-winning articles shall be discussed separately.

### ANNOUNCEMENT OF WINNERS AND PRESENTATION OF AWARDS

Announcement of the winners will be made in March 1993 in the Nihon Keizai Shimbun. With the exception of the commendation awards, abstracts of award-winning entries will be published in the Nihon Keizai Shimbun, and the articles will be published in full in the Nikkei Science magazine. A ceremony will be held for the presentation of awards (except commendation awards) to the winning entrants.
**ADDRESS FOR APPLICATIONS/INQUIRIES** :
21st-Century Earth Awards Secretariat 3—1—4—1004 Tsukiji, Chuo-ku, Tokyo 104, Japan Tel. : 03—3545—6897 Fax. : 03—3545—9769

# AMERICAN ASSOCIATION FOR CANCER RESEARCH

## 84th Annual Meeting

### Michael B. Sporn, Program Chairperson

### Orange County Civic Center, Orlando, FL

### May 19-22, 1993

## *Plenary Sessions and Symposia: Titles and Chairpersons*

**Impact of Molecular Biology on Cancer: Its Detection, Prevention, and Treatment** - Joint AACR/ASCO Session - Stephen H. Friend

**Transgenic Mice as Models for Cancer Pathogenesis** Owen N. Witte

**From Bench to Clinic: Concepts in the Design and Targeting of New Chemotherapeutic Agents** Bruce A. Chabner

**Invasion, Metastasis, and Angiogenesis** Robert S. Kerbel

**Mechanism of Action of Chemopreventive Agents: Basic Science and Clinical Applications** Martin Lipkin and Anita B. Roberts

**Topics in Drug Resistance** June L. Biedler

**Advances in Molecular Epidemiology of Human Cancer** Curtis C. Harris

**Cytokines in the Immunomodulation of Cancer** Elizabeth A. Grimm

**The Biology and Therapeutic Application of Normal Hematopoietic Stem Cells** Malcolm A. S. Moore

**The Restoration of Normal Function to Preneoplastic and Neoplastic Cells: Strategies for Differentiation Therapy** Waun Ki Hong

**Nitric Oxide and Superoxide as Endogenous Mediators of Carcinogenesis** Steven R. Tannenbaum

**The Biology and Pathogenesis of Prostate Cancer** Maarten C. Bosland

**Vitamin D and Cancer** Michael B. Sporn

**Stromal-Epithelial (Paracrine) Influences on Neoplasia** Gerald R. Cunha

**Proteases and Carcinogenesis** Lynn M. Matrisian

**Strategies for Utilization of Tumor-specific Antisense Molecules or Ribozymes for the Control of Tumor Growth** Jack S. Cohen

**Biology and Genetics of Human Preneoplastic Lesions** Walter N. Hittelman

**Human DNA and Protein Adduct Dosimetry: Assessment of Risk for the Development of Primary and Secondary Cancers** Regina M. Santella

**Biology and Treatment of Pediatric Cancer** Richard J. O'Reilly

**Latest Advances in Tumor Suppressor Genes** Edward E. Harlow

**Immunologic Approaches to Targeted Therapy** Ira Pastan

**Molecular Basis for the Modulation of Radiation Sensitivity** Gillies S. McKenna

**Gene Rearrangements in Cancer** Stanley J. Korsmeyer

**Protein Phosphatases in Carcinogenesis** Claude B. Klee

**Protein Kinase C: Modulation of the Cancer Phenotype and Target for Chemotherapy** Peter M. Blumberg

**The EGF and FGF Receptor Superfamilies: Recent Advances in Ligands, Receptors, and Signal Transduction** Andrew Baird and Michael Klagsbrun

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***Abstract Deadline: November 25, 1992***

Further Information: AACR Office • Public Ledger Building • 620 Chestnut Street • Suite 816 • Philadelphia, PA 19106-3483
**TELEPHONE** (215) 440-9300 • **FAX** (215) 440-9313

## IMMUNOTOXICOLOGY AND IN VITRO POSSIBILITIES

19-21 September 1993

Stockholm, Sweden

Details from the Meeting Secretariat, Lena Odland, CFN, Ministry of Agriculture, S-103 33 STOCKHOLM, Sweden. (Tel: +46 8 763 39 68; Fax: +46 8 10 93 39).

(W0155)S

WORKSHOP

# EMBO
## WORKSHOP ON GREEN AND HELIOBACTERIA

**Nyborg, Denmark · 16-19 August 1993**

### MAJOR TOPICS:

Chlorophyll Synthesis: Chlorosomes and Antenna Complexes; Energy Transfer; Reaction Centers; Electron Transport; Carbon, Hydrogen, Nitrogen and Sulfur Metabolism; Phylogeny, Ecology and Newly Discovered Organisms; and Molecular Genetics.

### INVITED SPEAKERS:

T.J. Aartsma (Leiden), J. Amesz (Leiden), N.G. Abdulaev (Moscow), C.A. Abella (Girona), S.I. Beale (Providence), R.E. Blankenship (Tempe), D.A. Bryant (University Park), R. Feick (Martinsried), U. Fischer (Oldenburg), A. Freiberg (Tartu), GG. Fuchs (Ulm), R.C. Fuller (Amherst), T. Gillbro (Umeå), G. Hauska (Regensburg), A.J. Hoff (Leiden), A.R. Holzwarth (Mülheim), D.J. Kelly (Sheffield), E.N. Kondratieva (Moscow), M.T. Madigan (Carbondale), K. Matsuura (Tokyo), M. Miller (Odense), M. Mimuro (Aichi), W. Nitschke (Freiburg), T. Nozawa (Miyagi), J. Oelze (Freiburg), J.M. Olson (Odense), J.G. Ormerod (Oslo), B.K. Pierson (Tacoma), H.V. Scheller (Copenhagen), R. Sirevåg (Oslo), K.M. Smith (Davis), W.S. Struve (Ames), W. Vermaas (Tempe), and D. Zannoni (Bologna).

In addition to the 34 invited speakers listed above there will be about 21 places (room and board included) reserved for younger research workers. Applications should include a c.v., a list of publications, a synopsis of current research, and a few recent reprints. Applications should be sent to

**Prof. John M. Olson, Institute of Biochemistry,**
**Odense University,**
**Campusvej 55,**
**DK-5230 Odense M,**
**Denmark**

***by 1 March 1993. Preference will be given to scientists from countries in the member states of the European Molecular Biology Organization (EMBO).***

(W0150)V

SYMPOSIA

## *SECOND BIENNIAL SYMPOSIUM ON BIOGEOCHEMISTRY OF WETLANDS*

***To be held at the Hilton Hotel, Baton Rouge, LA, 22-24 February 1993***

Sponsored by the Wetland Biogeochemistry Institute, Louisiana State University. Topics to be included will be: the role of wetlands in global climate change, the use of wetland biogeochemical processes to characterize regulatory wetlands, toxic heavy metal chemistry in wetlands, reactions of toxic organics in wetlands, the role of wetlands in improving water quality, plant-soil interactions in wetlands, and nutrient cycling in wetland ecosystems. Persons interested in making a presentation or attending the symposium should contact William H. Patrick, Jr. or Karen Gros, Wetland Biogeochemistry Institute, Louisiana State University, Baton Rouge, LA 70803. Telephone: 504-388-8810. Fax: 504-388-6423.

(NW8478)M

**INSTITUTE FOR BIOMEDICAL SCIENCE**
**Division of Molecular Pathology**

## FIRST ANNUAL SYMPOSIUM ON THE MOLECULAR PATHOLOGY OF HUMAN DISEASE

## *"FROM GENETICS TO GENE THERAPY"*

**UNIVERSITY COLLEGE LONDON MEDICAL SCHOOL**
**14 DECEMBER 1992**

To celebrate the opening of the Institute, and the formation of the new Division of Molecular Pathology, leading experts will review the impact of molecular biological techniques on the understanding, diagnosis and therapy of a range of human diseases.

**SPEAKERS: M Greaves** (ICR, London) *Leukaemia*; **N Hastie** (Edinburgh) *Wilm's Tumour*; **D Lane** (Dundee) *p53 and Cancer*; **G Nabel** (Michigan) *Gene Therapy*; **J Polak** (RPMS, London) *Endocrine Tumours*; **J Scott** (RPMS, London) *Cardiovascular Disease*; **D Weatherall** (Oxford) *α-thalassaemia*; **R Weiss** (ICR, London) *AIDS*; **R Williamson** (St Mary's Hospital, London) *Cystic Fibrosis*.

**REGISTRATION FEE** of £50 includes lunch; reduced rate (£10) available for PhD/MD/undergraduate students. Full-fee delegates will also receive the hardback edition of 'From Genetics to Gene Therapy': the first volume in the series on *The Molecular Pathology of Human Disease*, containing contributions by all the speakers and published by βios Scientific Publishers Ltd, Oxford (RRP: £45).

**FURTHER DETAILS/REGISTRATION FORM** available from: **Professor D Latchman, Division of Molecular Pathology, UCLMS, 3rd Floor Windeyer Building, 46 Cleveland Street, LONDON W1P 6DB. Tel: 071-380 9343 or Fax: 071-387 3310.**

(9534)M

## TARGET NEUROSCIENTISTS THROUGH nature CLASSIFIED

Throughout October and November extra copies of the following issues of Nature will be distributed to delegates attending these international shows covering the field of neuroscience.

ISSUE DATE: 22 OCTOBER

**22ND ANNUAL MEETING OF THE AMERICAN SOCIETY FOR NEUROSCIENCE**

Anaheim, USA 25 — 30 October
COPY DEADLINE: 16 OCTOBER

ISSUE DATE: 5 NOVEMBER

**NATURE 15TH INTERNATIONAL CONFERENCE**

Boston, USA 12 — 13 November
COPY DEADLINE: 30 OCTOBER

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# Sixth European Congress on Biotechnology

## Florence, June 13-17, 1993

*Organized on behalf of the European Federation of Biotechnology*
*Congress Secretariat. c/o prof. Laura Frontali, Dept. of Cell and Developmental Biology, University of Rome, P le Aldo Moro 5, 00185 Rome — Italy*

## PRELIMINARY PROGRAMME

### OPENING AND GENERAL LECTURES

The challenges of biotechnology — C. de Duve (B)
The 3D structure and design of proteins for biotechnology — T.L. Blundell (UK)
Biotechnology for AIDS diagnosis and prevention — L. Montagnier (F)
Risk-safety-communication : an overview — F.E. Young (USA)
Biotechnology for developing countries — A. Falaschi (UNIDO)
Biocatalysis in organic synthesis — H.E. Schoemaker (NL)
Critical problems in bioprocess technology — J. Tramper (NL)
Gene pools and biodiversity — W. Arber (CH)
Strategies of biotechnological industries — J.C. van Suijdam (NL)
Biotechnology research and agroindustry, a Commission point of view — F. Rexen (EEC)
New vaccines — R. Rappuoli (I)

### SYMPOSIA

Rational design of novel biocatalysis — S.B. Petersen (N)
Biocatalysis in organic synthesis : the potentiality of lyases — W. van den Tweel (NL)
Chaperonins and protein folding — F.U. Hartl (USA)
Lipases structure and function — R. Veiger (F)
Secretion and glycosylation of heterologous proteins — A. Hinnen (CH)
Receptor engineering — P. Comoglio (I)
Biotechological applications of antisense oligonucleotides — E. Uhlmann (D)
Analysis of different genomes of biotechnologial interest — A. Goffeau (B)
Genetic stability in engineered microogranisms — R. Fleer (F)
Biotechnology of lactic acid bacteria — M. Sibakov (SF)
Recent developments in nitrification/denitrification — J.G. Kuenen (NL)
Extremophiles in biotechnology — M. Rossi (I)
Biotechnology of filamentous fungi — J.R. Kinghorn (UK)
Yeast physiology and genetics — C Gancedo (E)
Physiological and engineering problems linked to immobilized cells fermentations — P. Linko (SF)
Physiology and genetics of anaerobes — G. Gottschalk (D)
Taxonomical aspects in biotechnology — K.H. Schleifer (D)
Impacts of genetic enginering on bioprocessing — M. Uhlén (S)
New separation methods in biotechnology — J.A. Asenjo (UK)
Affinity based processes in bioseparation — Y. Clonis (GR)
Antibody engineering — G. Winter (UK)
New developments in genetic diagnosis — S Ottolenghi (I)
New developments in immunodiagnostics — A. Albertini (I)
Modelling of bioprocesses — G. Bastin (B)
On line measurements of bioprocesses — A Lübbert (D)
Dynamics of bioreactors — L. Harvey (UK)
Integrated bioprocessing — K. Biedefmann (DK)
Scale-up of heterogeneous bioprocesses — J. Feijen (NL)
Multiphase bioreactors — M. da Fonseca (P)
Standardization in biotechnology — B. Ager (B)
Biocarriers and drug delivery — A. De Flora (I)
Biosensor design — R.D. Schmid (D)
Quantitative flow microfluorimetry in biotechnology — F. Srienc (USA)
Biotechnology of blood coagulation and fibrinolytic factors — W.D. Schleuning (D)
Enzymes an enzyme inhibitors as therapeutic agents — G. Rotillo (I)
Genetic engineering for the production of antibiotics — J.F. Martin (E)
Genetic and physiological approaches for the production of aminoacids — R. Kramer (D)
Expression of proteins in transgenic animals — L.M. Houdebine (F)
Biotechnological production of fine chemicals — E.J. Vandamme (B)
Biotechnological production of energetic sources — J. Miyaka (J)
Transgenic plants and crop productivity — F. Salamini (D)
New developments in biological control — D.H.L. Bishop (UK)
Biofertilizers and biopesticides — F. O'Gara (IRL)
Biotechnological innovations in food preservation, processing and analysis — T. Benitez (E)
New developments in the production of alcoholic beverages — M. Kielland-Brandt (DK)
Marine biotechnology — C. Gudin (F)
Agroindustrial wastes utilization by genetically engineered microorganisms — E. Martegani (I)
Biohydrometallurgy — P. Bos (NL)
Gaseous effluents treatment using biotechnological approaches — K. Kirchner (D)
Biomediation — W. Fritsche (D)
Ecologically sustainable processing technology — M. Narodoslawski (A)
Bioinformatics in biotechnology — C. Saccone (I)
Gene expression in animal cell cultures — A.T. Bull (UK)
Expression of heterologous genes in yeast systems — J.R. Shuster (USA)

### SPECIAL SYMPOSIA

Education and training in biotechnology — D. de Nettancourt (EEC)
Multimedial communications in biotechnological training — J. Hilton (UK)
Biosafety research in the EEC — M.P. Nuti (I)
Science Parks for biotechnological development — R. Knight (D)

### ROUND TABLES

"Biotechnology strategies for a Europe without frontiers"
"Science, public perception and biotechnology regulations"
"Intellectual property and litigation".

### WORKSHOPS

The list of WORKSHOPS will be defined after receiving the participation cards containing the tentative title of the posters it is planned to arrange between 30/40 WORKSHOPS.

### SCIENTIFIC AND TECHNICAL EXHIBITION

The exhibition will take place at the historical complex *"Fortezza da Basso"* and will be open throughout the Congress.

### CALL FOR ABSTRACTS

Congress participants are invited to contribute to the Scientific programme by
- a poster
- a symposium presentation
- a contribution to a workshop.

The abstracts, if accepted, will be presented as posters. Chairpersons of the Symposia or of the Worshops may invite some authors to give an oral presentation of their papers.

### PROVISIONAL TIME SCHEDULE

*November 1, 1992*
Deadline for submission of abstracts
*By January 1, 1993*
Authors will be informed about abstracts acceptance
*February 1, 1993*
Deadline for early registration
*June 13-17, 1993*
ECB6 Congress.

If you wish to receive the Preliminary Programme with the Registration and Abstract Forms or information on the Technical Exhibition, please contact:

**ECB6**
**c/o Organizzazione Internazionale Congressi**
**Via La Marmora 24**
**50121 Florence — Italy**
**Phone +39 55 5000631 — Fax +39 55 5002278**

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November 12-13th, 1992
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**CONFERENCE SESSIONS**

**I. Neuroscience in Medicine**
**II. Sensation: Vision, Hearing, Smell**
**III. Neuronal Death**
**IV. Development and Plasticity**

To Register contact:
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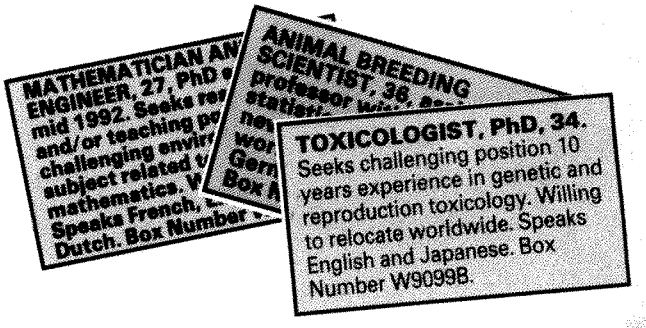


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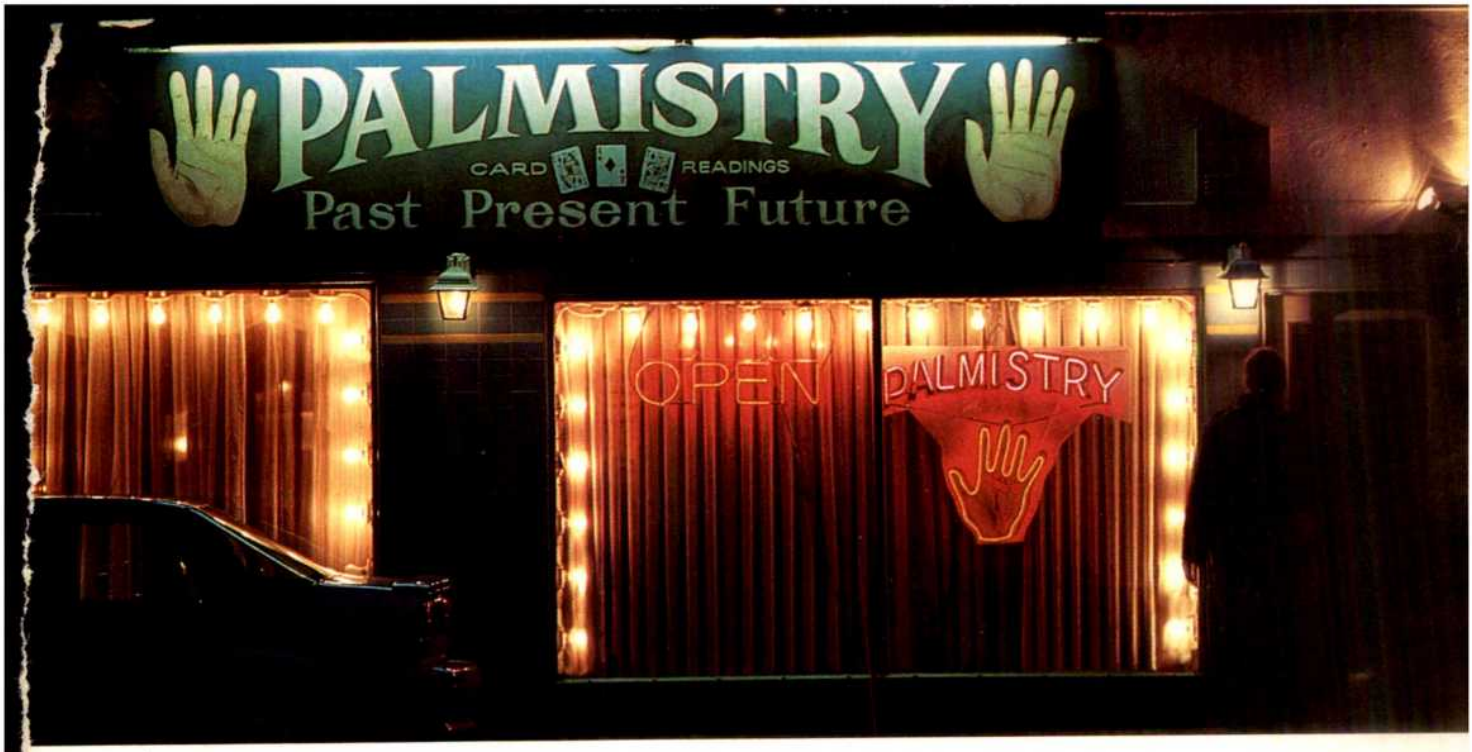


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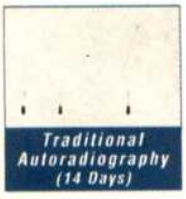



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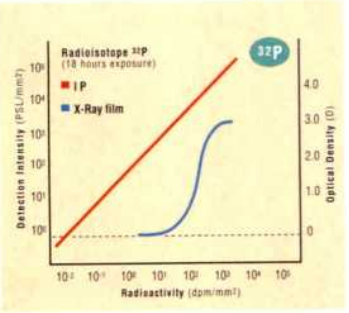


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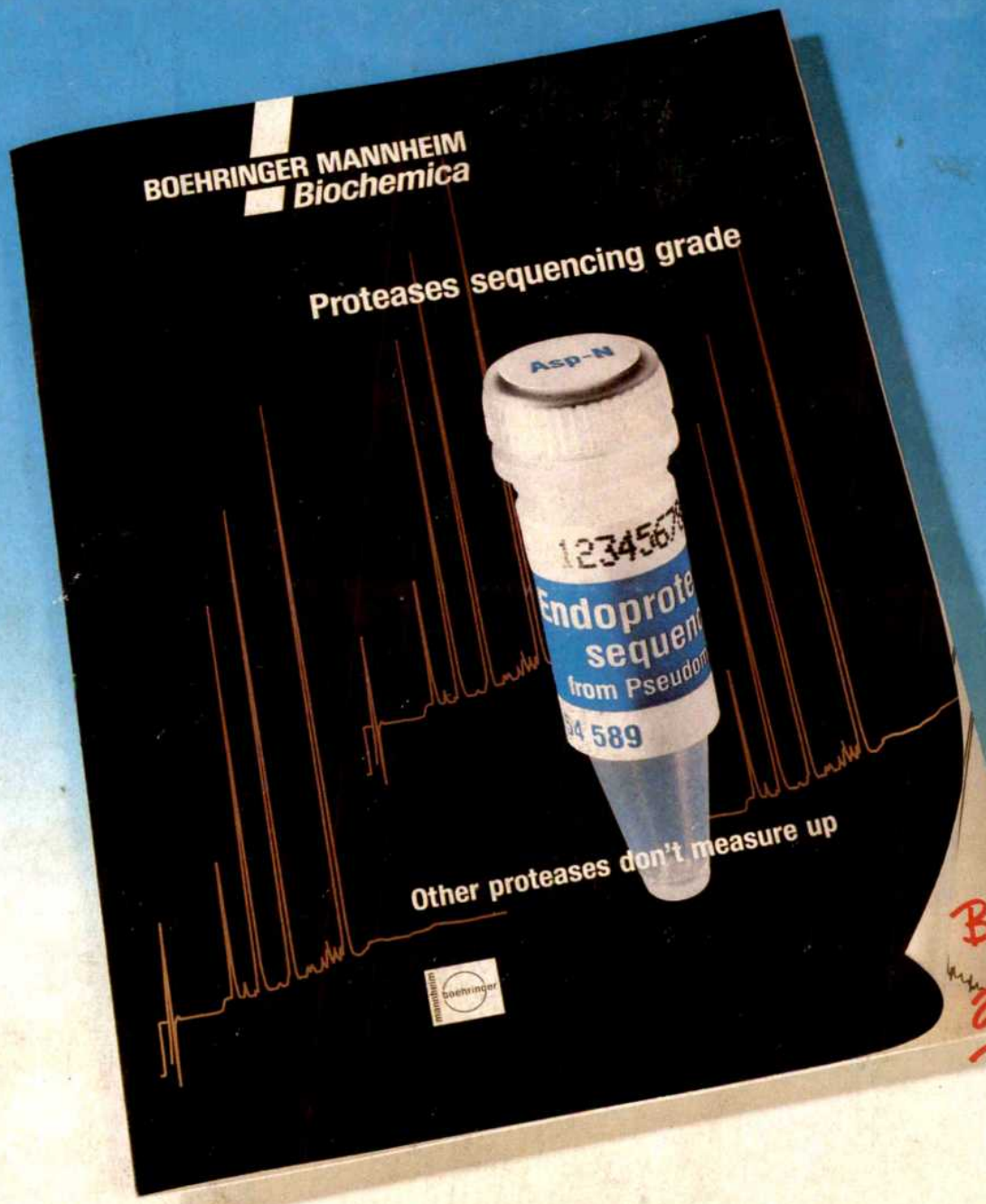


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